Science Library

Volume 91 Number 3

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University
H. B. STEINBACH, Washington University
Managing Editor

DECEMBER, 1946

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

BOOKS—WAR VICTIMS

DURING THE WAR, the libraries of half the world were destroyed in the fires of battle and in the fires of hate and fanaticism. Where they were spared physical damage, they were impoverished by isolation. There is an urgent need —now—for the printed materials which are basic to the reconstruction of devastated areas and which can help to remove the intellectual blackout of Europe and the Orient.

There is need for a pooling of resources, for coordinated action in order that the devastated libraries of the world may be restocked as far as possible with needed American publications. The American Book Center for War Devastated Libraries, Inc., has come into being to meet this need. It is a program that is born of the combined interests of library and educational organizations, of government agencies, and of many other official and non-official bodies in the United States.

The American Book Center is collecting and is shipping abroad scholarly books and periodicals which will be useful in research and necessary in the physical, economic, social and industrial rehabilitation and reconstruction of Europe and the Far East.

The Center cannot purchase books and periodicals; it must depend upon gifts from individuals, institutions, and organizations. Each state will be organized to participate in the program through the leadership of a state chairman. Other chairmen will organize interest in the principal subject fields. Cooperation with these leaders or direct individual contributions are welcomed.

WHAT IS NEEDED: Shipping facilities are precious and demand that all materials be carefully selected. Emphasis is placed upon publications issued during the past decade, upon scholarly books which are important contributions to their fields, upon periodicals (even incomplete volumes) of significance, upon fiction and non-fiction of distinction. All subjects—history, the social sciences, music, fine arts, literature, and especially the sciences and technologies—are wanted.

WHAT IS NOT NEEDED: Textbooks, out-dated monographs, recreational reading, books for children and young people, light fiction, materials of purely local interest, popular magazines such as *Time, Life, National Geographic,* etc., popular non-fiction of little enduring significance such as Gunther's *Inside Europe,* Haliburton's *Royal Road to Romance,* etc. Only carefully selected federal and local documents are needed, and donors are requested to write directly to the Center with regard to specific documents.

HOW TO SHIP: All shipments should be sent PREPAID via the cheapest means of transportation to THE AMERICAN BOOK CENTER, C/O THE LIBRARY OF CONGRESS, WASHINGTON 25, D. C. Although the Center hopes that donors will assume the costs of transportation of their materials to Washington, when this is not possible reimbursement will be made upon notification by card or letter of the amount due. THE CENTER CANNOT ACCEPT MATERIAL WHICH IS SENT COLLECT. Reimbursement cannot be made for packing or other charges beyond actual transportation. When possible, periodicals should be tied together by volume. It will be helpful if missing issues are noted on incomplete volumes.

Vol. 91, No. 3 December, 1946

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

A STRONGLY INTERSEXUAL FEMALE IN HABROBRACON

P. W. WHITING

University of Pennsylvania, Philadelphia, and the Marine Biological Laboratory, Woods Hole

In the parasitic wasp *Habrobracon juglandis* (Ashmead), diploid males have never shown any tendency toward intersexuality; they are as definitely male as their normal haploid brothers. When a "diploid male with female genitalia" was found, it was therefore regarded with especial interest. The specimen, designated freak 994, developing from a heavily x-rayed (29,300 *r*) egg, occurred among the offspring of a treated wild type (stock 33) female crossed with an untreated lemon honey male (Experiment by A. R. Whiting, 1945).

Freak 994 shows the heterozygous condition of the semidominant body color gene lemon inherited from its father. (Note light base of antennae in Figure 1.) The number of its antennal segments and its large ocelli are male characteristics. It was to be expected, therefore, that male reproductive reactions would occur. Several tests at different times failed to evince any response toward females although the specimen appeared healthy, drank honey water and lived for several days until fixed in Carnoy fluid. Since it likewise failed to give any response (female) to caterpillars, its indifference was probably not due to its sex type but to some unknown factor.

Because of the small "feminized" genitalia on the "male" body, freak 994 was at first recorded as a "diploid gynandroid male." Gynandroids, however, have always been haploids. They are mosaic males in which the two sexually different types of male tissue react in a complementary way to feminize the external genitalia (Whiting, Greb, and Speicher, 1934). Their mosaicism is shown by their asymmetry, not only in body color, in number of antennal segments, in mutant traits, and often in wing length, but especially in the external genitalia which are a mixture of normal male and feminized male structures with much reduplication and irregularity. In freak 994 there are no male genital structures and the female genitalia, consisting of a pair of sensory gonapophyses with no visible sting, are symmetrical and larger than in gynandroids. They are much smaller, however, than the female genitalia found in gynanders which are male-female mosaics with clearly separated male and female regions. That freak 994 is not a sex mosaic is shown by its symmetry in body coloration, in antennal flagella with nineteen segments in each and in length of wings and legs.

Two types of intersexes have hitherto been reported in Habrobracon. (1) Gynoid, dependent upon a single mutant gene, is a weakly intersexual male, functioning normally as a male, but having certain external traits, including antennae, feminized. (2) Nine intersexual females were reported (Whiting, 1943) occurring in a single fraternity. "Superficially, these appear to be the reverse of the gynoid

males, being more masculine anteriorly, feminine posteriorly." They resemble freak 994 in head and thorax and in the anterior part of the abdomen which are altogether like those of the male. In the posterior region, however, the sclerites are thickened, there is a normal sting and the sensory reproductive appendages are of full length characteristic of the female. "The nine intersexual females must be

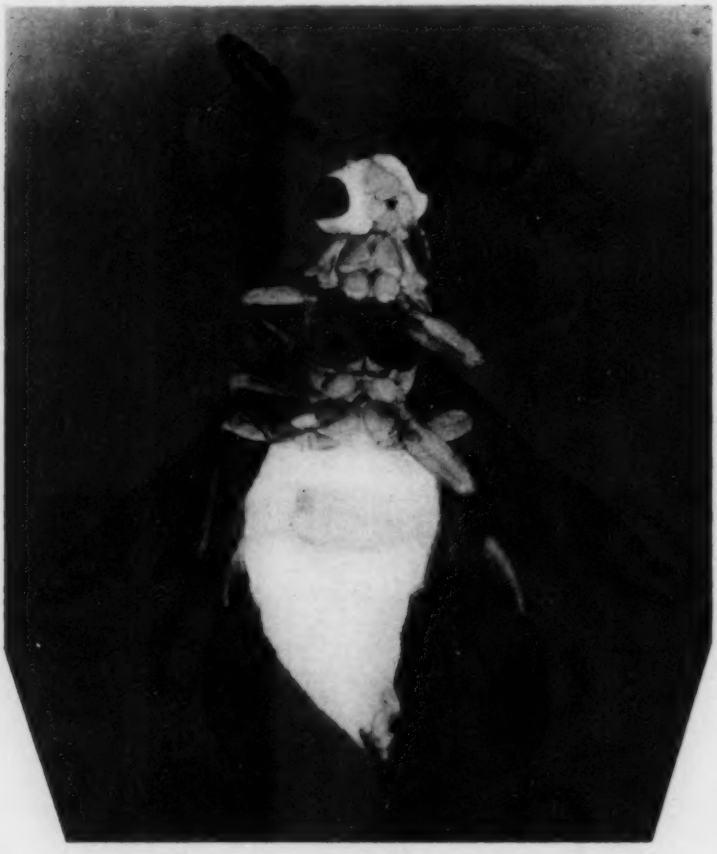


FIGURE 1

regarded as more strongly intersexual than gynoid males since antennae, ocelli and instincts are completely sex reversed." Freak 994 is an intersexual female; comparable to these nine but still more strongly intersexual because of greater restriction of the "female" region and reduction of the genitalia.

In Habrobracon, normal haploid males have cells almost as large as the corresponding cells of diploid females and in some stocks they are actually larger (Grosch,

1945). Cells of diploid males are much larger than are those of females or of haploid males. These relationships have been determined by counts of microchaetae within a given area on the upper surface of the wings, each microchaeta corresponding to a single cell. Study of the dispersion of microchaetae in freak 994 showed its cell size to be within the range for the female or haploid male and therefore much smaller than that characteristic of the diploid male. The marked shift of the intersex in the male direction does not then affect the size of its cells. It may be fundamentally female, heterozygous for the sex factor. This condition perhaps prevents the abnormal expansion of cell size while permitting development of antennae and ocelli of normal male type.

The nine intersexual females previously reported had internal abdominal structures as in the female with normal poison sac and glands and seminal receptacle. Each ovary, however, appeared to be a pair of sacs of oogonia showing no differentiation of nurse cells and ova. Serial sections were made of the abdomen of freak 994 and the internal structures were studied. The digestive tract is entirely normal with the crop greatly distended from honey water feeding. A poison apparatus is present but imperfectly developed and situated near the median plane, directly dorsal to the compound posterior nerve ganglion instead of being shifted laterad to the digestive tract. The poison glands are normal although of somewhat small size. Their ducts converge to a common duct connecting distally with an imperfect poison "sac" and proximally traversing the very short distance to the region where normally lies the root of the sting. The poison "sac," of approximately normal length, is reduced in diameter to an irregularly sclerotized strand. It is surrounded by longitudinal muscles as in a normal female. Nothing corresponding to a seminal receptacle could be located, nor were any gonads to be found. The fat body appears normal, surrounding the digestive tract and the poison apparatus dorsally and laterally.

Discussion

In the report on the nine intersexual females, it was suggested that they might be accounted for by a dominant mutation in a sex allele changing *xb* to xb^m. The intersexes would then be modified females, xa/xb^m. A similar hypothesis would cover freak 994, but here the mutation may have been x-ray induced and more potent than in the previous case so that the intersexuality would be more extreme with turning-point earlier in development.

Failure to find gonads in freak 994 does not necessarily mean that they were lacking from the beginning for they may have begun development and then disintegrated.

Comparison may be made between freak 994 and certain types of "deficient" individuals previously reported in Habrobracon (Whiting, 1926). Some of the "deficient" had external genitalia lacking but gonads present. Others had testes of reduced size, or present on one side, lacking on the other. Some of the "deficient" females with no trace of poison apparatus had well differentiated ovaries with eggs and nurse cells. This is just opposite to the condition found in the intersexual female, freak 994. There was no intersexuality among the "deficient."

Summary

An intersexual female developed from a heavily x-rayed egg fertilized by an untreated sperm. The specimen is more strongly intersexual than a group of nine previously reported, for its external female genitalia are much reduced, its poison apparatus defective and its ovaries altogether lacking. Externally, it appears like a diploid male with small female genitalia.

It is suggested that the x-radiation may have caused a change within a sex-differentiating allele, so that the heterozygote would develop into an intersex rather than a normal female.

LITERATURE CITED

Grosch, D. S., 1945. The relation of cell size and organ size to mortality in Habrobracon. *Growth*, **9**: 1–17.

Whiting, P. W., 1926. Influence of age of mother on appearance of an hereditary variation in Habrobracon. *Biol. Bull.*, **51**: 371–385.

Whiting, P. W., 1943. Intersexual females and intersexuality in Habrobracon. *Biol. Bull.*, **85**: 238–243.

Whiting, P. W., Raymond J. Greb and B. R. Speicher, 1934. A new type of sex-intergrade. *Biol. Bull.*, **66**: 152–165.

LOCI OF ACTION OF DDT IN THE COCKROACH (PERIPLANETA AMERICANA)

J. M. TOBIAS AND J. J. KOLLROS *

University of Chicago Toxicity Laboratory† and the Department of Physiology

In the cockroach, DDT produces symptoms which clearly reflect involvement of the neuromuscular apparatus. These are qualitatively much the same in all arthropods which have been studied, though there are important quantitative differences. Thus, in any given animal the time course of the poisoning is a function of dose, and for a dose of comparable toxicity in terms of final mortality, the symptoms unfold and death occurs much more rapidly in some insects (the fly) than in others (the roach) (Tobias, Kollros, and Savit, 1946a). In the roach, the sequence of symptoms is initiated by hyperextension of the legs, elevation of the center of gravity and development of postural instability. The hyperextension then decreases and is superseded by increasing and generalized tremulousness, involving the head, body, and all appendages; the gait becomes ataxic, and minor stimuli of sound or touch result in great hyperactivity, exhibited mainly in running and climbing. The animal falls on its back time after time until finally it can no longer right itself. Leg movements continue in the supine insect with two components, a high frequency intermittent tremulousness and a slower incoordinated flexion and extension. These two types of activity possibly reflect the double innervation which has been described for cockroach muscle (Pringle, 1939), one fiber type producing relatively slow tonic contractions, the other producing relatively fast twitches. It will be seen later that after poisoning these two types of movement can be independently altered. Activity finally diminishes progressively. The fast tremors disappear first and finally there remain only occasional isolated movements of body wall, tarsi, palpi, cerci, or antennae. When no further somatic movement can be detected, the heart usually continues to beat for some time, and electrical stimulation of the nerve cord may still evoke muscle responses. The animal may live in this condition for a day or so and finally die.

Mammals exhibit a similar symptomatology up to a point. In the rat and dog, given DDT intravenously or orally, muscular fibrillations and excessive blinking are followed by tremulousness, ataxia, falling and gross convulsive seizures. The animal may have a number of convulsions and die in the tonic phase of one or recover after gradual subsidence of symptoms. There is no period of prostration and nearly complete immobility as in the insect, because death occurs when systematic respiratory movements cease. In the insect, the small amount of body movement and twitching sufficiently augment diffusion for respiratory exchange. Then too, the insect is far more resistant to anoxia than is the mammal (Wigglesworth, 1939).

* Department of Zoology and The College. Present address, Zoology Department, University of Iowa.

† This work was carried out under contract with the Medical Division of the Chemical Warfare Service.

The frog, as might be expected, responds more like the insect than the mammal (Tobias, Kollros, and Savit, 1946b). Respiratory exchange through the skin can sustain life, and, after a period of hyperirritability, the animal lies prostrate and more or less immobile. Such symptomatology has prompted a number of investigations designed to discover a locus of action of DDT. As will be seen, there probably are a number of sites of action depending largely on dosage, but this point of view was only gradually attained.

In mammals (Crescitelli and Gilman, 1946), DDT apparently does not act directly on either muscle, myoneural junction or spinal cord. Since tremors persist after decerebration and mesencephalic transection, and since abnormal cerebral and cerebellar electrical activity persists after atlanto-occipital transection, neither cerebral cortex nor basal ganglia can be a critical site of action, and intact spinal afferents are obviously not necessary for the central effect. The cerebellum is considered, by these authors to be the most likely critical site of action in the mammal. Locus of action has also been investigated in insects. In Drosophila (Bodenstein, 1946), DDT seems not to act on muscle or myoneural junction, but does act on peripheral nerve and may act on the central nervous system. In the cockroach (*Periplaneta americana*), DDT has been found to act on nerve in high concentrations (Yeager and Munson, 1945), and, in low concentrations, on peripheral receptors (probably proprioceptors) (Roeder and Weiant, 1946). The latter workers also have evidence which they interpret to mean that high concentrations may act directly on either the myoneural junction or muscle itself. In the crab (*Cancer irroratus*) there is evidence for action on motor nerves (Welsh, 1946).

It was the purpose of this study to further investigate loci of action of DDT in an insect. Because of its large size and ready availability, the cockroach (*Periplaneta americana*) was used throughout.

Methods

Cockroaches were immobilized by exposure to 100 per cent CO_2 for 20–60 seconds or by etherization. After CO_2, anesthesia seldom lasted over a minute. Once anesthetized, the roach was fastened to a bit of cardboard by pins passed through either side of the pronotum. Appendages were held in any desired position by pins crossed over the body.

Decapitation was easily achieved by simply cutting the neck with a small scissors. The exposed stump was sealed with low melting-point paraffin. Ligation of the neck prior to decapitation to prevent loss of hemolymph did not prolong survival time. Such animals live about 60 hours (Table I).

To expose a thoracic ganglion, the spinasternum just caudal to the ganglion was cut through, and the incision extended along the sides of the sternal plate. After the plate was reflected forward, removal of superficial fatty tissue and tracheal tubes fully exposed the ganglion. The connectives anterior to the ganglion were held in a jeweler's forceps and cut with iridectomy scissors. Traction on the connectives exposed the lateral nerves, which were sectioned. Finally, the posterior connectives were cut and the ganglion removed. Simple isolation of a ganglion from the rest of the nerve cord can be achieved without excising it by cutting the connectives through slits in the cuticle. Complete transection of the entire roach between sets

TABLE I

Effect of lesions of the central nervous system on symptoms of DDT poisoning in the cockroach

Operation		No. roaches			Average survival, hours	General results	Legs which showed DDT effects
		Controls, no DDT	Operated				
Nature	Site (See Fig. 1)		Before DDT	After DDT		Hyperactivity, tremors and convulsions	
Decapitation	At A	14	—	—	61	Rare tremors in 3 animals—no convulsive activity	None
		—	14	—	56	Typical DDT effects in all animals	All
		—	—	14	56	Typical DDT effects in all animals	All
Transaction of ventral nerve cord	At C and D (both anterior and posterior to thoracic ganglion no. 2)	16	—	—	104	None in any animals	None
		—	15	—	60	Typical DDT effects in all animals	All in 13 animals; 2nd and 3rd pairs in two animals
Destruction of ganglion	Th. 2 (thoracic ganglion no. 2)	15	—	—	77	None in any animals	Leg 2 paralyzed in all
		—	15	—	71	Typical DDT effects in all animals	1 and 3 in 13 animals. 1, 2 and 3 in two animals

of legs results in an isolated segment containing a ganglion, nerves and the attached legs. Such a preparation, if kept moist, is viable for at least 6 to 8 hours.

Excision of the heart largely prevents circulatory removal of substances applied to structures to elicit a local effect. Longitudinal incisions through the cuticle, on either side of the heart tube along its entire length, isolate a strip whose removal carries the heart with it. The heart may be cauterized with equal ease (Yeager and Munson, 1945).

Methods for the administration of measured doses of DDT to insects have been described elsewhere (Tobias, Kollros, and Savit, 1946a).

RESULTS

Localization experiments with uncontrolled DDT doses

Except where otherwise specified, contact poisoning was carried out by confining the roach for 5–15 minutes within a glass cylinder coated with DDT previously precipitated from acetone solution.

Roaches decapitated before or after such contact with DDT behaved like intact poisoned animals (Table I). Therefore, neither the supra- nor the sub-oesophageal ganglia are essential for the development or maintenance of DDT-induced motor activity in the legs or body. Ventral nerve cord connectives were transected both anterior and posterior to the mesothoracic ganglion (Fig. 1, levels C and D). Animals so prepared but given no DDT showed incoordination of the mesothoracic legs when walking, but there were no symptoms which could be confused with those of DDT poisoning. When such animals were subsequently poisoned, however, the mesothoracic as well as the other legs exhibited typical abnormal activity (Table I). After complete transection of the whole body of the poisoned roach, at both these levels (excised segment Fig. 1), leg tremulousness and hyperactivity continued un-

abated in the isolated segment. The application of DDT emulsion or DDT in acetone to the cut surface of such segments obtained from normal roaches evoked typical DDT effects in the attached legs within a few minutes. The same was true of DDT applied directly to the exposed ganglion in the otherwise intact animal. Emulsion or acetone without DDT had no such effect.

The cells of origin of the leg nerves lie within the lateral halves of the thoracic ganglia, each ganglion in the adult being formed by the midline fusion of two embryonic ganglion masses. Median sagittal section of the ganglion in a poisoned roach (Fig. 1, level F) did not stop hyperactivity in either of the legs innervated

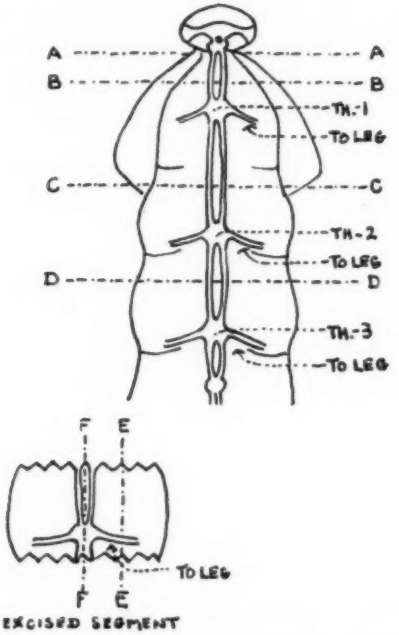


FIGURE 1. Levels of section in cockroach nervous system.

from the resulting ganglion halves. Therefore, even half a segment contains all the structures necessary for the maintenance of DDT symptoms in a leg.

If, however, the entire ganglion was removed the results were generally quite different. Mesothoracic ganglia were removed from thirty normal roaches. The corresponding legs of all were paralyzed and failed to respond to touch or pressure. Shortly after the operation, fifteen of the animals were contact poisoned. All showed typical DDT effects in the pro- and metathoracic legs, but the ganglionectomized mesothoracic legs remained entirely quiet in thirteen and showed only occasional tarsal twitching and some slight movement of the other joints in two. Similarly, ganglionectomy after the development of hyperactivity, rather than before

TABLE II

Experiments on isolated roach segments containing local ganglion, nerves, and legs

No. of segments	Material applied	Route	Number of segments in which there was persistent DDT leg activity
6	Nothing	—	None
3	Emulsion* without DDT	On cut surface	None
2	Acetone without DDT	Injected	None
7	1 Per cent DDT emulsion*	On ganglion	Occurred in all
4	10 Per cent DDT in acetone	Injected into vicinity of ganglion	Occurred in all
3	DDT powder	On ganglion	Occurred questionably in one

* Emulsion—1 per cent DDT, 10 per cent peanut oil, 1 per cent lecithin and 88 per cent 0.90 per cent NaCl (5).

poisoning, either stopped or markedly reduced symptoms in the corresponding legs (Table IV). As was to be expected from these experiments, section of leg nerves lateral to the ganglion stopped or markedly reduced activity in many (65 per cent) of the legs (Table III).

These experiments tentatively suggested that the ventral cord ganglion was critically involved in the motor action of DDT and might itself be a site of action. Conflicting data, however, were also obtained. It was possible, as also reported by others (Yeager and Munson, 1945; Roeder and Weiant, 1946), to produce motor

TABLE III

Visible effect of DDT on amputated legs
(Dose not controlled)

No. of legs	Source of legs	Treatment after amputation	Results after amputation
30	Normal roaches	Normal controls	No spontaneous movement
58	DDT poisoned roaches tremulous and hyperactive	—	Continued activity in 20. No movement in 38
12	Normal roaches	Emulsion without DDT injected into cut end	No movement in any
22	Normal roaches	Emulsion with 1 per cent DDT applied to cut end	Movement in 1, others all quiet
35	Normal roaches	Emulsion with 1 per cent DDT injected into cut end	Movement in 25, other 10 quiet
9	Normal roaches	Acetone without DDT injected into cut end	Movement in 1, other 8 quiet
13	Normal roaches	Acetone with DDT injected into cut end	Movement in 3, other 10 quiet

activity in a large percentage of amputated legs by the injection of DDT emulsion (1 per cent DDT, 1 per cent lecithin, 10 per cent peanut oil, and 88 per cent 0.9 per cent NaCl solution). It will also be recalled that ganglionectomy failed to entirely quiet the legs in two of fifteen experiments (Table I).

Such conflicting data were difficult to interpret. Ganglionectomy or denervation usually stopped leg activity, but this was not invariably the case, and it was possible to produce activity in the amputated legs by injection of DDT. It was suspected that such results might be resolved in terms of DDT dose. Further experiments were then done with measured doses of DDT.

Localization experiments with controlled doses of DDT

It was immediately found that the effectiveness of ganglionectomy in abolishing motor effects was inversely related to dose (Table IV). That is, as the dose of DDT was increased ganglionectomy stopped movement in progressively fewer cases.

TABLE IV

Effect of ganglionectomy on symptoms after various doses of DDT

No. experiments	DDT*	Results of ganglionectomy	
		Number resulting in complete cessation of activity	Number resulting in a reduction of activity
10	Usual moderate contact dose (5–10 mins. in DDT coated tube)	70%	30%
5	Excessive contact dose (approximately 2 hours in DDT coated tube)	20%	80%
25	5–30 mg. DDT per kg. injected intra-abdominally in emulsion**	68%	32%
12	60–70 mg. DDT per kg. injected intra-abdominally in emulsion**	50%	50%
15	130 mg. DDT per kg. injected intra-abdominally in emulsion**	7%	93%

* LD-50 for DDT injected intra-abdominally in emulsion is 20 mg. per kg. (Tobias, Kollros, and Savit, 1946a).

** Emulsion—1 per cent DDT, 1 per cent lecithin, 10 per cent peanut oil, 88 per cent of 0.9 per cent NaCl.

In all cases, however, even when movements were not entirely stopped they were both qualitatively and quantitatively changed. The high frequency tremulousness was always markedly reduced or entirely abolished and the slower movements were much diminished.

Nicotine, in low concentrations, is known to block synaptic transmission centrally as well as peripherally (Libet and Gerard, 1938; Pringle, 1939) but not axonal transmission. When applied to the cockroach ganglion there is an initial burst of electrical hyperactivity (100–800 impulses per sec.) followed by electrical silence (Pringle, 1939). As would be expected, such application of nicotine to a ganglion also produces great motor hyperactivity in the attached leg which can be abolished by amputating the leg (Yeager and Munson, 1945).

Now then, if nicotine applied to a ganglion in a concentration which did not

affect peripheral nerve were to stop DDT symptoms, this would be added evidence for the importance of the ganglionic cell bodies or synapses in the development and maintenance of such symptoms. After poisoned roaches became hyperactive the heart was excised. This did not decrease activity, but served to greatly diminish circulatory transport of solutions applied for local effects. Solutions were then applied as small droplets to the ganglion or a region of leg nerve exposed by cuticle excision.

Dilute nicotine solutions (0.01 per cent in insect Ringer) applied to the leg nerves of the normal or poisoned roach did not paralyze the leg. Typical DDT induced activity could not be stopped in this fashion. This was almost surely not due to failure of nicotine to reach the nerve since spontaneous movement as well as that following electrical stimulation of the ganglion could be stopped by a similar

TABLE V

Effect of locally applied nicotine and novocaine on motor symptoms of DDT poisoning after various doses of DDT*

No. experiments	DDT**	Number of experiments in which activity was modified			
		1.0% novocaine	0.01% nicotine		
		Injected into tibia	Injected into tibia	Applied to ganglion	
		Complete inactivity	Complete inactivity or reduced activity	Activity stopped	Activity reduced
4	10–30 mg. per kg. applied to body surface 18 hours before	100%	0%	100%	0%
9	100 mg. per kg. applied to body surface 18 hours before	100%	0%	77%	22%
13	500 mg. per kg. applied to body surface			23%	77%
4	1000 mg. per kg. applied to body surface			0%	100%

* All experiments on cardiectomized roaches to prevent circulatory removal of substances applied for local effect.

** DDT applied to surface in acetone. LD-50 for DDT so applied is 10 mg. per kg. (Tobias, Kollros, and Savit, 1946a).

administration of 1 per cent novocaine. When, however, this nicotine solution was applied to a ganglion (in the same normal or poisoned animal in which it was ineffective on peripheral nerve) there was a short-lived burst of great activity in the legs of the segment, followed by complete immobility or markedly decreased activity. Since the nicotine was effective in concentrations which did not block peripheral nerve, it was concluded that it was acting by blocking ganglionic synapses and not by spill-over to the emerging nerve roots. It is clear (Table V) that, as in the case of ganglionectomy, the immobilizing effect of nicotine decreased as the dose of DDT increased, and, as was also true after ganglionectomy, if nicotine did not stop activity it considerably decreased and modified it.

Discussion

It is clear that DDT can produce motor symptoms by effects peripheral to the ganglion. It is equally clear, however, that the ganglion plays a role in the initiation and maintenance of symptoms and that this role is to some extent dependent upon DDT dose.

Roeder and Weiant (1946) found that, in the cockroach, very low concentrations of DDT can initiate centripetally directed, high frequency (300–400 per sec.), temporally irregular bursts of nerve impulses, presumably excited by action of DDT on the campaniform sensilla (presumptive proprioceptors). There was no evidence of any muscle movement which might have initiated such centrally directed impulses. Welsh (1946) has demonstrated that DDT in very low concentrations can also favor repetitive response of motor fibers (*Cancer irroratus*) to a stimulus normally evoking single responses, and Yeager and Munson (1945) have concluded that high concentrations can produce similar changes in the cockroach.

The results of ganglionectomy, in the cockroach (surgical or nicotine inactivated), after various doses of DDT, support the view that the initiation and continuation of the hypermotor symptoms of DDT poisoning after low doses of DDT require an intact sensori-motor reflex arc, and that random afferent impulses in sensory nerves may indeed, as suggested by Roeder and Weiant, excite motor neurones in the ganglion to initiate incoordinated muscular activity. From the experiments here reported, this would appear to be a part of the common sequence of changes in the roach poisoned by uncontrolled contact doses. The fact that ganglionectomy becomes less and less effective as the dose of DDT is progressively increased would suggest that larger amounts of DDT may act directly on motor nerves. Obviously, these data do not rule out a possible direct action on muscle. Within the dose ranges which have been used there is, however, no conclusive evidence for a direct action on muscle. This is not to say that such action could not occur at some sufficient dosage level. In addition, ganglionectomy is seen to have stopped the rapid tremulousness after any dose which was tried, suggesting that the high frequency movements may be initiated reflexly rather than by direct action on motor fibers at large as well as at low doses of DDT. This general picture is compatible with the subsidence of high frequency tremulousness before subsidence of slower muscular activity. If the former is reflex and the latter due to direct nerve action one might expect this order of dropping out on the basis of much greater fatigability for the reflex arc than for the nerve trunk.

Pattern development of symptoms

It has been claimed (Laüger, Martin, and Müller, 1944) that if DDT be put on one leg of a fly the development and progression of symptoms follow a definite, orderly and reproducible path from leg to leg. Such a phenomenon might be very important indeed for an understanding of the mechanism of DDT action. The authors have not been able to confirm this finding.

Conclusions

1. Neither decapitation, section of one or several nerve cord connectives nor complete transection of the entire insect body at one or several levels between nerve cord ganglia prevents the development of the typical motor effects of DDT in any

of the legs of the cockroach. After combined antero-posterior isolation of a nerve cord ganglion, even median sagittal section of the ganglion does not prevent motor symptoms in the legs still attached to the lateral ganglionic cell masses. Therefore, the anatomical elements necessary for development of the motor symptoms of DDT are contained within the lateral half of a body segment which contains the lateral half of a ganglion, leg nerves and peripheral structures.

2. Since the motor symptoms of DDT poisoning can occur in amputated legs, in legs whose nerves have been cut, and in legs whose segmental ganglia have been destroyed, it is possible for DDT to produce its motor effects by action on some structure or structures peripheral to the segmental ganglion.

3. The motor symptoms of DDT poisoning can be stopped or diminished in a leg by ganglionectomy, leg nerve section, or ganglion synaptic block with nicotine. The effectiveness of these procedures is in inverse relation to the dose of DDT administered. These findings suggest that, in the cockroach, low doses of DDT may excite motor fibers reflexly by impulses fired into the ganglion over afferent nerve fibers, whereas high doses may act on elements on the motor side of the ganglion and thus not require an intact reflex arc. Since ganglionectomy stops the fast component of the hypermotor activity, however, equally well after large or small doses of DDT, this component may be reflexly initiated and maintained after all doses of DDT.

LITERATURE CITED

Bodenstein, D., 1946. Investigation on the locus of action of DDT in flies (Drosophila). *Biol. Bull.*, **90**: 148.

Crescitelli, F. and A. Gilman, 1946. Electrical manifestations of the cerebellum and cerebral cortex following DDT administration in cats and monkeys. *Amer. Jour. Physiol.*, **147**: 127.

Laüger, P., H. Martin and P. Müller, 1944. The constitution and toxic effect of botanicals and new synthetic insecticides. *Helv. Chim. Acta*, **27**.

Libet, B. and R. W. Gerard, 1938. Automaticity of central neurones after nicotine block of synapses. *Proc. Soc. Exp. Biol. and Med.*, **38**: 886.

Pringle, J. W. A., 1939. The motor mechanism of the insect leg. *Jour. Exp. Biol.*, **16**: 220.

Roeder, K. D. and E. A. Weiant, 1946. The site of action of DDT in the cockroach. *Science*, **103**: 304.

Savit, J., J. J. Kollros and J. M. Tobias, 1946. The measured dose of gamma hexachlorocyclohexane (γ 666) required to kill flies and cockroaches and a comparison with DDT. *Proc. Soc. Exp. Biol. and Med.*, **62**: 44.

Tobias, J. M., J. J. Kollros and J. Savit, 1946a. Relation of absorbability to the comparative toxicity of DDT for insects and mammals. *Jour. Pharm. and Exp. Ther.*, **86**: 287.

Tobias, J. M., J. J. Kollros and J. Savit, 1946b. Acetylcholine and related substances in the cockroach, fly and crayfish and the effect of DDT. *Jour. Cell. Comp. Physiol.* (In press.)

Welsh, J. H., 1946. Personal communication.

Wigglesworth, V. B., 1939. *The principles of insect physiology.* Dutton and Co., Inc., New York.

Yeager, J. F. and S. C. Munson, 1945. Physiological evidence of a site of action of DDT in an insect. *Science*, **102**: 305.

TILLINA MAGNA: MICRONUCLEAR NUMBER, ENCYSTMENT AND VITALITY IN DIVERSE CLONES; CAPABILITIES OF AMICRONUCLEATE RACES

C. D. BEERS

Department of Zoology, University of North Carolina, Chapel Hill

It is well established that the number of micronuclei in *Tillina magna* is highly variable. For example, Gregory (1909) found 6–10, and Ilowaisky (1921), in a ciliate which he called *Pseudocolpoda cochlearis cienkowskii*, reported 2–6. An examination of Ilowaisky's text and figures shows conclusively that his ciliate was *T. magna* Gruber (1879) as Kahl (1931, p. 282) pointed out. Kahl apparently regarded six as the typical number. Bresslau (1922) observed the nuclei in sufficient detail to note the extrusion of macronuclear material at division and the presence of several micronuclei, though he reported no counts of their actual number. The writer (1946), in a study dealing chiefly with the history of the nuclei during division and encystment, counted the number of micronuclei in 100 individuals (50 active and 50 encysted) of each of three clones, and found that it varied from 6 to 11 in one clone and from 4 to 6 in the other two. Thus the number was found to vary in different individuals of the same clone, and the mean number was found to vary in different clones. Active specimens and resting cysts of any particular clone had on the average like numbers of micronuclei. Contrary to statements in the literature, it was shown that the micronuclei divide at the time of cell division, and not indiscriminately or without regard to cell division. The mechanism by which two daughter cells may receive unlike numbers of micronuclei at division, thus accounting for variations in number within a clone, was described.

The significance of the wide variation in micronuclear number is unexplained. Structurally and physiologically an individual having only 4 micronuclei does not appear to be fundamentally unlike one having 11 micronuclei. The same condition prevails in the closely related species, *T. canalifera*, which I was formerly disposed to regard (1945) as identical with *T. magna*. However, on the basis of information furnished me by Dr. George W. Kidder, of Amherst College, it appears that *T. canalifera* merits recognition as a valid species, chiefly because of the very conspicuous nature of its canal system. In *T. canalifera*, Turner (1937) reported 4–14 micronuclei, though Burt, Kidder, and Claff (1941), in specimens obtained from the late Dr. Turner, found only one. Hence, it is clear that the micronuclear number may vary from 1 to 14, yet the evidence indicates that the uninucleate and multinucleate races were equally cultivable, vigorous, and capable of producing normal resting cysts.

The present study of *T. magna* was undertaken in order to obtain additional information concerning two points: (1) the normal variation in micronuclear number in various natural races and (2) the significance of such variation. The investigation of the first point is readily feasible, in that the micronuclei may be counted with absolute certainty in Feulgen preparations of favorably oriented resting cysts or

medium-sized trophic specimens. The investigation of the second point, though less suited to direct approach, is not impracticable. A number of questions arise, some of which submit to experimental analysis. For example, is the number of micronuclei related in any way to size, whether of trophic specimens, division cysts or resting cysts; to division rate; to vitality, meaning capacity to endure with undiminished vigor as generations pass; to ability to produce resting cysts; to the viability of such cysts; or to ability to excyst? Of the foregoing measurable characters, the following were selected as being most readily amenable to experimental investigation: ability to produce resting cysts, size and viability of such cysts, capacity to excyst, division rate and vitality. These then, will be considered in relation to micronuclear number, though not all of them will receive equal consideration. The study assumed unlooked-for interest when it became evident that three of the races were amicronucleate. Thus a comparison of the potentialities of micronucleate and amicronucleate clones became possibile.

Materials and Methods

Twenty clones of *T. magna*, to be designated numerically, were used in the study. The progenitors of these respective clones were collected in a meadow, known locally as Sparrow's Pasture, in the vicinity of Chapel Hill, North Carolina. Comparisons with clones from other sources were desirable, but unfortunately attempts to collect *Tillina* elsewhere in the Chapel Hill region, and in the vicinity of Stanford University, California, and Woods Hole, Massachusetts, were unavailing. In this study a clone refers to all the progeny which were derived asexually from a single resting cyst or trophic specimen. The intervention of encystment and subsequent excystment is not considered to be a valid reason for changing the clonal designation, since there is no evidence that encystment in *Tillina* involves a sexual process which might change the genetic constitution of the clone. (It should perhaps be recalled that *Tillina*, like its near relative *Colpoda*, reproduces within a thin-walled temporary cyst, from which usually four progeny emerge shortly as a result of two successive divisions. The term encystment, as used in this study, does not refer to these temporary division cysts, but to the protective or resting cysts.) It is not definitely established that all of the twenty clones were genetically different, since their histories prior to their period of laboratory life were unknown.

The progenitors of clones 1, 2, 6, 8, 9, 11, 12, 13, 15, 17, 18, and 19 were taken as active specimens on Sept. 10, 1945, and these clones were therefore cultured simultaneously in the early part of the study. Eight of the foregoing clones, namely, 6, 11, 13, 18, 15, 19, 1, and 17, have already been reported on briefly under the numerical designations 1 to 8, respectively (Beers, 1946a). In the present paper my original numerical designations of all clones have been changed for the convenience of the reader in using the accompanying tables. The progenitors of clones 3, 14, 16, and 20 were isolated on Feb. 4, 1946, when dried leaves and debris, after 8 months of storage at 19° C., were immersed in weak hay infusion; these clones were therefore cultured simultaneously. It is evident that they were derived from dried cysts. The progenitors of clones 4, 5, 7, and 10 were isolated on April 8, 1946, when moist leaves and debris, which had recently washed against the bases of willow saplings in the meadow, were immersed in hay infusion; these clones were

maintained in culture simultaneously toward the end of the study. They were undoubtedly derived from wet cysts.

An attempt was made to maintain each of the clones in pure-line culture for a period of 60 days. Sixteen of the clones were readily cultivable and continued with undiminished vigor throughout the period; four were intractable in that their division rates declined and the lines encysted well before the end of the period. Thus the laboratory histories of the clones varied, although the conditions of culture were uniform. The details, in relation to micronuclear number, will follow.

Each clone was cultured in depression slides in the form of four sub-lines. These were maintained at 23° C. in 0.05 per cent lettuce infusion to which suitable quantities of *Pseudomonas fluorescens*, grown on nutrient agar, were added as food. Previous experience has shown that this general procedure, combined with daily isolations and transfers to fresh environments, meets adequately the cultural needs of *Tillina* (Beers, 1944, 1945). Records were made daily of fission rates and other points of interest.

Surplus animals from the lines were stained on cover glasses by the Feulgen method in order to make micronuclear counts of active specimens. Small stock cultures of each clone furnished precystic specimens when the food supply neared depletion. These specimens were removed and allowed to encyst on cover glasses in the manner described by Beers (1946). Thus convenient preparations were available, first for making measurements of living cysts, and then for Feulgen staining. All measurements and micronuclear counts of cysts were made on *single* resting cysts. These are the common type. They are practically spherical and therefore well suited for making accurate measurements.

Normal Variation in Number of Micronuclei

The data bearing on diversity in micronuclear number in the twenty clones are summarized in Table I, in which the clones are arranged and numbered in the order of decreasing mean numbers of micronuclei. The data, ignoring for the present the mean diameters of resting cysts, are largely self-explanatory. A few points deserve special mention.

In any particular clone both active specimens and resting cysts showed practically the same extremes of variation (range) in micronuclear number and had essentially the same mean number of micronuclei.

In different clones the mean numbers of micronuclei were extremely variable. Some clones (e.g., 1 and 2) had consistently high mean numbers; others (e.g., 16 and 17), consistently low numbers, with many intergrades between these extremes.

Clones 18, 19, and 20 were amicronucleate. This statement is not based on casual observation, but on an intensive study of these clones. In trophic specimens and resting cysts the micronuclei of *Tillina* are not disposed toward secretiveness. They are never imbedded in the macronucleus. Each has an endosome which stains intensely and conspicuously by the Feulgen method. In mature resting cysts only the rod-shaped or ellipsoid macronucleus and the micronuclei stain to any appreciable extent; there is nothing in the cytoplasm to conceal the micronuclei. In trophic specimens it is true that the food vacuoles also stain, but the micronuclei always lie in the clear peri-macronuclear space and are not in a position to be concealed by the vacuoles. Moreover, considerable numbers of individuals of clones

18, 19, and 20 were stained. These included not only the usual resting cysts and medium-sized trophic specimens, but also young cysts, cysts in the process of excystment, and individuals just excysted. None showed a micronucleus, whereas individuals of the remaining seventeen clones, stained at the same time by the same method, invariably showed micronuclei.

The individuals of some clones (e.g., 1, 3, 5, 12) showed great diversity in micronuclear number within the clone. This fact is brought out clearly by the range which is cited for these clones, and it is further emphasized by the high standard deviations in the clones. Clone 12 showed the greatest degree of heterogeneity in that the range in micronuclear number extended from 2 to 11, with all intervening numbers being represented. On the other hand, some clones (e.g., 2, 4, 8; 13, 15, 16, 17) were relatively homogeneous, with narrow ranges and low standard deviations. Other clones lay between these extremes. Only the amicronucleate clones showed complete homogeneity.

Thus, it is seen that individuals of a clone exhibit varying numbers of micronuclei, that clones differ with respect to their mean number, and that amicronucleate clones exist in nature.

The mean number of micronuclei in the 850 micronucleate active specimens of Table I (representing 17 clones) was 7.06; the mean number in the 850 micronucleate cysts was 7.08. Unfortunately, the number in the progenitor of each clone

TABLE I

Tillina magna. *Variation in number of micronuclei in twenty clones; relation of micronuclear number to size of cysts. The clones are numbered and arranged as the mean number of micronuclei (average of means for fifty active specimens and fifty resting cysts) decreases.*

Numerical designation of clone	Range in number of micronuclei		Mean number of micronuclei ± standard deviation		Mean diameter of 50 resting cysts in microns ± standard deviation
	50 active specimens	50 resting cysts	50 active specimens	50 resting cysts	
1	10–16	9–16	12.90±1.87	12.32±1.93	85.76± 9.65
2	8–12	9–12	10.48±0.94	10.96±0.87	82.94± 7.65
3	7–14	6–13	9.62±2.30	9.24±2.08	79.68± 8.72
4	7–10	7–10	8.52±0.82	8.60±0.95	88.62± 7.25
5	6–14	6–12	8.17±1.95	8.28±1.66	88.36± 9.61
6	6–10	6–10	7.56±1.28	7.78±1.24	93.60± 6.21
7	5–10	5–10	7.25±1.28	7.38±1.36	92.44± 7.08
8	6– 9	6– 9	7.16±0.85	7.06±0.92	78.50± 7.82
9	5– 9	4– 9	6.52±1.15	6.74±1.21	84.16± 8.62
10	4– 8	4– 8	5.98±1.52	5.94±1.46	85.42± 5.28
11	5– 8	5– 9	5.90±1.03	5.76±0.96	86.14± 5.34
12	2–10	2–11	5.74±2.41	5.90±1.97	85.64± 5.32
13	4– 6	4– 6	5.12±0.42	5.10±0.45	80.64± 8.92
14	4– 8	4– 8	5.12±1.51	4.98±1.42	84.18± 4.76
15	4– 6	4– 6	4.86±0.53	5.20±0.57	81.60± 8.41
16	4– 6	4– 6	4.92±0.63	4.98±0.75	80.92± 7.37
17	3– 5	3– 5	4.28±0.75	4.14±0.74	91.42± 6.87
18	—	—	0	0	86.28± 5.63
19	—	—	0	0	84.32±10.36
20	—	—	0	0	81.52± 5.34

is unknown, since the micronuclei cannot be identified in living specimens. It seems reasonable to assume that the progenitor of each had a number approximately equivalent to the mean which was determined for the clone, and that it produced some offspring having fewer, and some having more, than its own number.

It is well known that the number of micronuclei is variable in many species of ciliates. Thus, *Paramecium multimicronucleatum* has 2 to 7 (Powers and Mitchell, 1910), though usually 4 (Wenrich, 1928); *Spathidium spathula*, 6 to 9 (Maupas, 1888, p. 247); *Urostyla grandis*, 10 to more than 40 (Tittler, 1935); and *Stentor coeruleus*, 10 to 42 within a single clone (Schwartz, 1935). On the whole, such variations within a species appear to have little effect on the structure or behavior of the individuals and to be without functional significance. This general conclusion is supported by the observations on *T. magna* which follow immediately.

Number of Micronuclei in Relation to Various Aspects of Cystment

All the clones produced normal resting cysts upon the depletion of the food supply in small stock cultures prepared with surplus animals from the lines. Furthermore, all the specimens in such cultures encysted; none persisted in prolonged swimming, thereby to perish of starvation. Hence, it is clear that the ability to encyst is not dependent on the presence of the micronucleus, since amicronucleate as well as micronucleate clones were able to encyst. Moreover, the cysts of all the clones remained viable for many months. They could be activated at any time after the fourth day by immersion in distilled water or 0.05 per cent lettuce infusion. From 2 to 2.5 hours were required for emergence at 23° C., and practically 100 per cent of the specimens excysted. No precise figures are given here, since the percentage of excystment under various conditions has been dealt with in a previous paper (Beers, 1945) and the present study contributes nothing new on this point. However, the present results show clearly that the viability of resting cysts and their capacity to excyst are in no wise related to the presence of a micronucleus, or to the number of micronuclei. Well over 90 per cent of the cysts produced in the various clones were single ones; double cysts appeared sporadically, some in amicronucleate clones, some in micronucleate. Amicronucleate cysts undergo the usual colpodid type of macronuclear reorganization, involving the extrusion of a portion of the macronuclear substance into the cytoplasm (Taylor and Garnjobst, 1941; Burt, Kidder and Claff, 1941; Beers, 1946).

The size of the cysts in different clones was made the subject of special study, for it was thought that the number of micronuclei might affect the size of the cysts. The diameters of fifty living single cysts of each clone were measured, each measurement extending from the outer surface of the ectocyst of one side to the corresponding surface of the other. The results of these measurements are included in Table I. An inspection of the table shows at once that the calculation of coefficients of correlation between micronuclear number and cyst size would be of little value, since cyst size is independent of micronuclear number. For example, if we consider certain extremes in micronuclear number, it is seen that the cysts of clone 1 had a mean diameter of about 85 μ, and those of clone 19 a diameter of 84 μ, with approximately equivalent standard deviations. Clones 2 and 20 and clones 4 and 18 constitute other examples of extreme disparity in micronuclear number with close agreement in cyst size. Among the micronucleate clones, other examples of

wide divergence in micronuclear number, yet with general uniformity in cyst size, are furnished by clones 3 and 16 and by clones 6 and 17. On the other hand, some clones having widely divergent micronuclear numbers produced cysts of dissimilar sizes, e.g., clones 3 and 17 and clones 6 and 13. Thus it is seen that clones having widely different micronuclear numbers may produce cysts of equivalent mean sizes or of dissimilar mean sizes. It must be concluded that there is no relation between the number of micronuclei and the size of the cysts.

The same conclusion is reached if we adopt another approach and consider cyst size in clones which had similar, or only slightly different, micronuclear numbers. For example, clones 6 and 7 had similar mean numbers of micronuclei and they produced cysts of equivalent mean sizes; clones 15 and 16 constitute a second example. On the other hand, clones 7 and 8 had similar micronuclear numbers, but they produced cysts which differed significantly in mean size; clones 16 and 17 furnish another example. Hence, clones having similar mean micronuclear numbers may produce cysts of equivalent mean sizes or of different mean sizes. Again, it is evident that there is no relation between number of micronuclei and size of cysts. The mean diameter of the 850 micronucleate cysts of Table I was 85.23 μ; that of the 150 amicronucleate cysts was 84.04 μ.

A word concerning the size of individual cysts may be of interest. In any particular clone of *T. magna*, whether micronucleate or amicronucleate, there is usually wide variation in cyst size, even though the cysts under immediate consideration are all produced in the same small stock culture—meaning a Columbia culture dish containing 1 cc. of fluid. Cysts in such a culture often vary in size from 75 μ to 95 μ; extremes of 64 μ and 104 μ have been noted. The factors which affect cyst size appear to be of a complex physiological nature and are therefore not readily identifiable.

The point of greatest interest in this consideration of various aspects of cystment is the fact that amicronucleate and micronucleate clones behaved alike; clearly the micronucleus of *T. magna* plays a negligible role, if any, with reference to encystment, viability of cysts, excystment, macronuclear reorganization within the cysts, and cyst size.

Number of Micronuclei in Relation to Division Rate and Vitality

The cultural histories of the twenty clones are presented in Table II. Clones 1, 2, 3, and 5 could not be maintained in culture for the arbitrary period of 60 days. The remaining clones were maintained with undiminished vigor and were discontinued at the end of the period.

First, the division rates of the sixteen vigorous clones will be considered in relation to micronuclear number. Clones 4, 6, 7, and 8, as has been seen, had relatively high micronuclear numbers (means, about 7 to 8.5). The total average number of generations produced by the four sub-lines of these respective clones varied from 149 to 174. Thus the clones themselves varied with respect to mean division rate. Clones 9–12 had intermediate micronuclear numbers (means, 6 to 6.5); these clones produced from 149 to 167 generations. Clones 13–17 had low micronuclear numbers (means, 4 to 5); they produced from 160 to 176 generations. Thus clones having intermediate or low micronuclear numbers produced in general as many generations, and had therefore the same division rates, as clones having relatively high micronuclear numbers.

Amicronucleate clones 18–20 produced from 154 to 172 generations. Thus the amicronucleate clones had approximately the same division rates as the micronucleate clones; e.g., clone 19 produced almost as many generations as clone 4; clone 18 produced more than clone 10; clone 20 produced about as many as clone 9 or 13. Many kinds of comparisons are possible, and the reader may choose to make other comparisons between amicronucleate and micronucleate clones. For example, clones 18–20 had higher division rates (i.e., produced more generations) than clones 8, 10, and 14; but clones 18–20 had lower division rates than clones 4, 7, and 16. Thus the general conclusion that amicronucleate clones have the same division rates as micronucleate clones is not invalidated. Sections of amicronucleate division cysts showed that the macronucleus undergoes the usual reorganization after each of its divisions, as in normal micronucleate cysts (Burt, Kidder and Claff, 1941; Beers, 1946).

Next, the vitality of the sixteen vigorous clones must be considered. An examination of the number of divisions produced in the successive 5-day periods in any particular clone shows that the clone was dividing as rapidly at the end of the experiment as at the beginning. Within the time limits of the experiment, the clones showed no decrease in vitality as measured by the division rate. How long the sixteen clones would have continued without diminution in vitality is a question that cannot be answered on the basis of the available data. The important findings are these: Some clones which have relatively high micronuclear numbers are as vigorous as those which have low numbers; amicronucleate clones are fully as vigorous as many micronucleate clones.

Clones 1, 2, 3, and 5 must receive special consideration. As has been said, these clones could not be maintained in culture for the duration of the 60-day experimental period. Clone 1 showed a rapid decrease in fission rate and encysted on the fourth day. Some of the cysts were activated and new lines were established. These in turn declined shortly and encysted. Three additional attempts were made to culture clone 1; each time the lines encysted after 3–5 days. Indeed, clone 1 was so refractory that without these repetitions it would have been impossible to obtain sufficient specimens for the usual number of micronuclear counts. Clones 2, 3, and 5 likewise could not be maintained in culture for 60 days. Their histories are presented in sufficient detail in Table II. Following the encystment of the original lines of these clones, new lines were established with excysted specimens, but they also declined and encysted after 3–4 weeks of culture. It may be maintained that the decline and encystment of these four clones resulted from a failure to meet their cultural needs. However, the conditions of culture were adequate for a total of sixteen clones, and it seems not unreasonable to assume that they were likewise adequate for clones 1, 2, 3, and 5 and to conclude that these clones declined as a result of intrinsic factors.

It has been shown that clones 1, 2, and 3 had higher micronuclear numbers than any of the other clones. Clone 5 likewise had a high number, though slightly lower than clone 4, which was cultivable. The evidence indicates that a large number of micronuclei may be detrimental to the welfare of the organism and incompatible with high vitality, but the number of such clones studied was too small to justify a general conclusion. On the whole, the results show that in *T. magna* the rate of division and the vitality of the race are in no wise related to the number of micro-

TABLE II

Tillina magna. *Relation of number of micronuclei to division rate and vitality in twenty clones maintained in pure-line culture, usually for sixty days. Four clones having high micronuclear numbers encysted before the expiration of sixty days; the remaining sixteen continued with undiminished vigor, though their mean micronuclear numbers (see Table I) varied from 8.56 to 0.*

Successive five-day periods	Total average number of divisions per line for successive periods																			
	Cl. 1	Cl. 2	Cl. 3	Cl. 4	Cl. 5	Cl. 6	Cl. 7	Cl. 8	Cl. 9	Cl. 10	Cl. 11	Cl. 12	Cl. 13	Cl. 14	Cl. 15	Cl. 16	Cl. 17	Cl. 18	Cl. 19	Cl. 20
1	5.5*	13.5	11.5	14.5	15.5	12.5	16.0	11.5	12.5	11.5	13.0	13.0	14.5	14.5	14.5	14.0	12.5	11.5	12.5	13.5
2		11.0	9.5	16.5	12.5	13.0	15.0	12.5	11.5	10.5	14.0	14.5	15.0	13.0	12.5	14.0	11.5	12.5	13.5	12.5
3		9.5	12.5	13.5	11.5	14.5	12.5	12.5	13.0	12.5	12.5	15.5	13.5	12.5	13.0	15.0	12.5	16.5	14.5	11.5
4		7.5*	10.0	14.0	10.0	11.5	14.0	13.0	12.0	15.5	11.5	13.5	14.5	11.5	16.5	15.5	13.5	12.5	15.0	14.5
5			8.5	13.0	7.0	15.0	12.5	11.5	14.5	14.5	14.0	12.5	15.0	14.5	14.0	14.5	14.5	11.0	15.5	12.5
6			9.5	16.5	0.5*	12.5	14.5	11.5	15.0	13.5	13.5	13.5	12.5	12.5	15.5	16.5	13.5	13.0	16.5	14.5
7			7.8	15.0		14.0	15.0	12.5	15.5	12.5	12.5	14.5	14.5	13.5	13.5	14.5	14.0	13.5	14.5	15.5
8			2.2*	13.5		13.5	16.5	13.5	14.0	11.5	14.5	15.0	13.5	14.5	12.5	13.0	15.0	14.5	13.0	14.5
9				14.5		13.0	13.5	11.5	16.0	12.5	13.0	13.5	11.5	11.5	14.5	14.5	14.5	12.5	14.5	13.5
10				14.0		16.0	14.0	12.5	13.5	10.5	15.0	12.5	12.5	14.0	16.0	16.5	13.5	11.0	13.5	12.5
11				15.5		12.5	15.5	13.0	12.5	13.5	12.5	14.5	14.5	12.5	13.5	14.5	13.0	12.5	15.0	13.5
12				14.0		14.0	14.5	13.5	12.0	10.5	12.5	15.0	15.0	13.0	14.5	13.5	12.5	13.0	14.0	15.5
Total number of generations	5.5	41.5	71.5	174.5	57.0	162.0	173.5	149.0	162.0	149.0	158.5	167.5	166.5	157.5	170.5	176.0	160.5	154.0	172.0	164.0

*Cl. 1. Encysted after 4 days.
*Cl. 2. Encysted after 20 days.
*Cl. 3. Encysted after 37 days.
*Cl. 5. Encysted after 26 days.

nuclei, or even to the presence of a micronucleus, since amicronucleate clones proved to be as vigorous as micronucleate clones.

The origin of the amicronucleate races cannot be accounted for with certainty. It is agreed that such races may arise at any of four periods in the life of ciliates generally: at endomixis, by the transformation of all the reconstruction micronuclei into macronuclei; at autogamy or conjugation, by an analogous transformation of all the derivatives of the synkaryon or amphinucleus into macronuclei; or at division, by an unequal distribution of the micronuclei to the daughter cells. By studying dividing individuals of an unusual race of *Paramecium caudatum*, Wichterman (1946) observed the simultaneous production of bimicronucleate and amicronucleate daughters. In *T. magna* conjugation is of rare occurrence, and endomixis and autogamy are unknown. Therefore, it is likely that amicronucleate races usually take their origin in an unequal distribution of the products of division. However, it should not be concluded from the present results that 15 per cent of all *Tillina* clones are amicronucleate; actually such clones appear to be very exceptional. I have stained many specimens during a period of 8 years; all these specimens had micronuclei, except for the members of clones 18–20.

Discussion

The functional significance of the nuclear dimorphism of the Euciliata has long held the attention of protozoologists. In general, the dimorphic condition has been viewed as representing a segregation of idiochromatin and trophochromatin, the former in the micronucleus, the latter in the macronucleus. It was originally assumed, since ciliates normally possess both types of nuclei, that both are necessary for the continued survival of the individual. Although the precise functions of the respective nuclei are difficult to determine, two lines of investigation have supplied pertinent findings, namely, a study of the capabilities of amicronucleate ciliates of natural occurrence and a study of regenerative capacity, survival, and reproduction in ciliates which have been deprived experimentally of either nucleus, or of both, whether by merotomy or other operative procedures.

The existence of naturally occurring amicronucleate races has long been conceded by such authorities as Woodruff (1921), Calkins (1930), and Reichenow (1929, p. 29) and is now accepted as a fact. The potentialities of these races, as revealed by intensive laboratory study, have demonstrated that the micronucleus is not at all necessary for the maintenance of the essential vital processes of the individual, whereas the macronucleus is indispensable. Aside from the absence of a micronucleus and the manifest inability to carry to completion such processes as endomixis, autogamy and conjugation, amicronucleate races of many ciliates do not differ structurally or physiologically from micronucleate ones.

Thus, Dawson (1919; 1920) maintained an amicronucleate race of *Oxytricha hymenostoma* in pure-line culture for 289 generations (4 months) and in small mass cultures for 5 months longer. The absence of micronuclei did not prevent the animals from attempting to conjugate, but these attempts were abortive. Woodruff (1921) cultured amicronucleate races of *Oxytricha fallax* and *Urostyla grandis* for 246 and 128 generations, respectively, and maintained a race of *Paramecium caudatum* in pure-line culture long enough to determine that it was definitely amicro-

nucleate. A few pairs of conjugants occurred in mass cultures of *O. fallax*, but they failed to live when isolated.

Amicronucleate races of other ciliates have likewise been isolated and cultured long enough to demonstrate not only their viability but their sustained vigor and good health. Among these are the following: (1) *Spathidium spathula.* Moody (1912) was unable to find micronuclei in her specimens, though she was able to culture them for 218 generations. It is evident that they were amicronucleate, since the micronuclei of *Spathidium* were observed and counted by Maupas and were found regularly by Woodruff and Spencer (1922). (2) *Didinium nasutum.* Patten (1921) cultured an amicronucleate race, which was derived from an exconjugant of a normal micronucleate race, for 652 generations. Conjugation occurred in the amicronucleate race, but the exconjugants invariably died. The resting cysts were likewise inviable. It is evident that the race arose through the faulty reorganization of the exconjugant. (3) *Paramecium bursaria.* Woodruff (1931) cultured a race characterized by micronuclear instability for 7 years. Neither endomixis nor conjugation was observed. The race was originally bimicronucleate; later it was variable, exhibiting from 1 to 4 micronuclei; then for about 4 years it was unimicronucleate; finally, a derived race showed no micronucleus, although this race was apparently as healthy and vigorous as its bimicronucleate ancestors which were isolated 7 years earlier. Woodruff (p. 543) aptly points out that "whatever function the micronuclear apparatus plays, the somatic life of the animals is not obviously influenced by profound variations in volume or in distribution of micronuclear material." (4) *Urostyla grandis.* Tittler (1935) found amicronucleate individuals in stock cultures which previously contained only micronucleate specimens. They were indistinguishable externally from their micronucleate progenitors, and they flourished in mass cultures for 2 years. Their macronuclear divisions followed the usual complex pattern characteristic of the species. The race produced resting cysts, some of which could be excysted, although endomixis, which usually occurs in the precystic forms, was absent. Evidently some of the cysts were not entirely normal, since they showed a tendency to disintegrate, perhaps because of the omission of endomixis. (5) *Colpoda steini.* Piekarski (1939) studied comparatively the structure and reproduction of a micronucleate and an amicronucleate race and was able to culture the latter for approximately 6 years. Both races were equally cultivable and vigorous. They reproduced within division cysts from which four progeny regularly emerged and they produced normal resting cysts. They showed the same sequence of events in the division of the macronucleus. These events are of special interest, in that eight chromatic (Feulgen-positive) bodies appear in the macronucleus of the young division cyst. Ultimately each of the daughters receives two of them, and thus the behavior of these bodies suggests an equational distribution of chromosomes. Piekarski concludes that the absence of a micronucleus had no recognizable effect on the activities of the animals.

The present study of amicronucleate races of *Tillina magna* likewise demonstrates the adequacy of the macronucleus, not only for long-continued reproduction accompanied by sustained vigor, but also for encystment and excystment. Thus endowed with the ability to produce viable resting cysts, these races would seem to be capable of indefinite survival, even under the changeable conditions of a natural environment.

Still more remarkable, in connection with the capabilities of amicronucleate races, are the observations of Sonneborn (1940) and Kimball (1941) on mating types. In certain races of *Paramecium aurelia* Sonneborn found a small percentage of animals which, upon undergoing autogamy or conjugation, developed a new macronucleus from a fragment of the old macronucleus. Since the micronuclei commonly disappeared in clones produced by these animals and since the mating type never changed at macronuclear reorganization, Sonneborn concludes that "hereditary characters (including mating type) of clones from macronuclear regenerates cannot be directly determined by micronuclei, for they persist in the absence of micronuclei. Mating type must be determined by the macronucleus. . . ." Kimball was able to assign amicronucleate specimens of *Euplotes patella* to definite mating types, since they paired readily with individuals of known mating type. He concludes that "the micronucleus is thus unnecessary for an animal to be of a definite mating type."

With reference to the role of the nuclei in the regeneration of ciliates following merotomy, the results obtained with different species are not always in complete accord. The physical properties of the cytoplasm constitute an experimental variable; a fluid cytoplasm or a rigid pellicle may interfere with the closure of an injury and thus affect regeneration adversely. Balamuth (1940) has presented an excellent review of the extensive literature on this subject. For the present only a few representative examples of regeneration in ciliates will be considered, with special reference to the nuclear components of the merozoa (cell fragments).

It is well known that enucleate fragments of ciliates can neither regenerate nor continue to live, whereas nucleate fragments regenerate successfully and pursue normal lives. These conclusions are particularly evident in Balamuth's six-page tabular summary of the findings on regeneration in the ciliates. As a rule the macronucleus and micronucleus cannot be separated, and a nucleate fragment, as in Dembowska's work (1925) on *Stylonychia mytilus*, usually means one having both macronuclear and micronuclear material.

However, some investigators have succeeded in obtaining nucleate merozoa of the two types desirable for an experimental analysis of the role of the individual nuclei in regeneration; namely, macronucleate (without micronuclei) and micronucleate (without any part of the macronucleus). Reynolds (1932), for example, in work on an amicronucleate *Oxytricha fallax*, found that various types of macronucleate merozoa could regenerate their missing parts and resume their normal physiological activities. Schwartz, using microdissection techniques, was able to remove the entire macronucleus from *Stentor* and yet leave a number of micronuclei in the specimens. These micronucleate individuals never survived. By means of successive operations, he was also able to remove all the micronuclei from a few specimens, leaving a portion of the beaded macronucleus in place. These macronucleate individuals regenerated and could be cultured as pure lines. Thus he produced experimentally an amicronucleate race, which as regards size and division rate was not different from the normal controls. Bishop (1943), employing the ultra-centrifuge as a means of obtaining merozoa of *Oxytricha fallax*, obtained sixty-seven macronucleate fragments, all of which regenerated, and seven micronucleate fragments, none of which regenerated. Twelve of the regenerated macronucleate individuals were cultured as amicronucleate pure lines. Bishop concluded (p. 451) that "the lack of micronuclear material makes no difference in the regenerative capacity, division rate, motility or morphology of *Oxytricha fallax*."

On the other hand, there is evidence that in some forms the micronucleus, as well as the macronucleus, is necesesary for regeneration and survival. Thus Reynolds found that both nuclei are necessary for the regeneration of merozoa of *Euplotes patella*. This observation is in accord with the results of Taylor and Farber (1924), who, by means of a micro-pipette, removed the micronucleus from fifty specimens of *E. patella*, all of which died within a few days. None produced more than four progeny. Hence, these authors conclude that "the micronucleus plays more than a purely germinal role in the life history of *Euplotes patella*." However, the situation in *E. patella* is confused, for some of Kimball's unimicronucleate double animals produced viable amicronucleate individuals at division. Some of them survived as clones, though with a low division rate; one such amicronucleate clone survived for 341 days. The results show, according to Kimball (p. 30), "that the micronucleus is not essential for continued life in at least some clones of *Euplotes patella*, though its absence results in a marked decrease in vigor." In various species of *Uronychia* (Young, 1922), and in *Uroleptus mobilis* (Tittler, 1938), *Spathidium spathula* and *Blepharisma undulans* (Moore, 1924) both types of nuclei appear to be necessary for the complete regeneration, growth and division of merozoa.

Thus the evidence afforded by the long-continued culture of a number of naturally occurring amicronucleate races demonstrates conclusively that the macronucleus alone suffices for the maintenance of the vegetative life of the organism—meaning by vegetative life such diverse activities as locomotion, food capture, digestion, assimilation, growth, excretion, respiration, reproduction, and maintenance of cell proportions and form. On the basis of this evidence the normal role of the micronucleus in vegetative life appears to be one of relative passivity. The evidence from operative procedures shows that in many ciliates the macronucleus alone is adequate for complete regeneration, as well as for subsequent growth and division, whereas in other ciliates the micronucleus also is necessary. There is no authenticated case on record in which the micronucleus alone is adequate for the maintenance of vegetative functions or for regeneration. Balamuth's thorough survey of the literature leads him to make the following comment in his summary: "Of the dual nuclear apparatus, only the macronucleus can be shown to function in the actual regenerative process. The role of the micronucleus in this connection is as yet unclear; apparently it is more important in the viability of some forms than in others." On the whole, the evidence tends only to emphasize the importance of the macronucleus and to attest to the validity of Calkins' comment (1930, p. 161) on this organelle: "Far from being negligible it is on the contrary probably the most important element of the cell in matters of metabolism, reorganization, and continued cell life."

The tendency to underestimate the importance of the macronucleus in the life of the organism results probably from its apparent monotony of structure. Lacking chromosomes, its division is usually unspectacular. Nevertheless, its mode of origin is not an incidental phenomenon in the life of the ciliate, for both macro- and micronucleus almost invariably have a common and simultaneous origin. Usually they develop from the synkaryon of the conjugant, by divisions which appear to be equational. Again, they develop from the synkaryon of autogamy or from the endomictic micronucleus. Thus they inherit equally from a common nucleus of origin, and each receives equivalent chromatin elements, chief among which are presumably the genes. In the definitive micronucleus these elements retain the ability to or-

ganize periodically as chromosomes, and thereby to arrest the attention of the observer. Once in the definitive macronucleus, on the contrary, they never again assemble in the form of chromosomes. However, it is possible that they are distributed at macronuclear division by a mechanism which is fully as effective as the mitotic distribution of chromosomes, though less conspicuous. For example, it is not impossible that they are represented in multiplicate in the macronucleus and distributed at random throughout its substance. Thus each daughter at division would be reasonably assured of receiving representatives of every type of chromatin element. The behavior and the potencies of the macronuclear fragments of Sonneborn's unusual specimens of *Paramecium aurelia* indicate a multiplicate representation of the chromatin elements. In these specimens the forty or more macronuclear fragments grew and segregated during subsequent cell divisions, until there was only one in each cell. Thus each fragment was adequate for the regeneration of a complete macronucleus and for the continued life of the organism, even in the absence of micronuclei. Hence, Sonneborn concludes that "the normal macronucleus must contain at least forty complete and discrete genomes." A more precise mechanism for the distribution of the chromatin elements, involving, for example, a differential streaming of genetically equivalent elements toward opposite ends of a polarized macronucleus, may be postulated. Regardless of the type of mechanism involved in the distribution of the chromatin elements of the macronucleus at division, the fact remains that inheritance in an amicronucleate ciliate is no less precise, to judge by the structure and physiological activities of the offspring, than in a micronucleate ciliate. The fact that the behavior of the macronucleus does not conform to the chromosome theory of heredity in *sensu stricto*, in that chromosomes are absent, may mean simply that a different mechanism for the distribution of the genes is involved.

Whether the macronucleus of amicronucleate ciliates may justifiably be regarded as an amphinucleus—one containing idiochromatin as well as trophochromatin, as Woodruff (1921), Moore and others have suggested—seems doubtful in the light of recent investigations. Thus it has been shown by Schwartz and by Bishop that viable amicronucleate races of *Stentor* and *Oxytricha* may be derived by experimental means from normal individuals in which the idiochromatin and trophochromatin were presumably segregated in the two types of nuclei. The macronucleus of these individuals, following removal of the micronuclei, was adequate to maintain all the usual vegetative activities in the derived amicronucleate races.

In conclusion, the evidence shows that the macronucleus is the essential nuclear element in the vegetative life of ciliates. The micronucleus functions largely, if not solely, in the periodic replacement of the macronucleus and in the production of new genetic combinations, some of which undoubtedly render the species better adapted to survival. The nature of the physiological conditions which call for a renewal of the macronucleus is not clear; that such renewal meets an imperative physiological need is shown by the widespread occurrence of the phenomenon in the Euciliata.

Summary

The number of micronuclei was examined in 50 trophic specimens and 50 resting cysts of each of 20 clones of *Tillina magna*, three of which were amicronucleate.

In any particular clone trophic specimens and resting cysts contained approximately equivalent mean numbers of micronuclei. In different micronucleate clones the mean number varied from 4.21 to 12.61. The mean for 1,700 specimens of 17 clones was 7.07.

The number in the individuals of any particular micronucleate clone was variable; some clones showed relatively little variation, e.g., 3 to 5 micronuclei; others, considerable variation, e.g., 2 to 11 micronuclei. The smallest number observed in any micronucleate individual was 2; the largest, 16.

All the clones produced normal resting cysts upon depletion of the food supply (*Pseudomonas fluorescens*). The cysts of different clones were equally viable and capable of excystment. Their size was unaffected by the number of micronuclei. Amicronucleate cysts showed the usual macronuclear reorganization. Hence, neither the number of micronuclei nor the absence of micronuclei affected encystment, viability and size of cysts, excystment or macronuclear reorganization.

An attempt was made to maintain each clone in pure-line culture for 60 days and thereby to examine the division rate and vitality. Four clones were refractory and encysted before 60 days expired. The remaining 16 clones, including the three amicronucleate ones, survived with undiminished vigor and were discontinued. The 13 micronucleate clones produced from 149 to 176 generations during the 60-day period; the three amicronucleate clones produced 154, 164, and 172 generations, respectively. Hence, the 16 surviving clones showed slight differences in their average daily division rates, but neither the divison rate nor the vitality of these clones was correlated with variations in micronuclear number or with the absence of micronuclei. Division cysts of amicronucleate clones showed the usual macronuclear reorganization after each division of the macronucleus. The four refractory clones had high micronuclear numbers.

Since conjugation is rare and endomixis and autogamy are unknown in *Tillina*, it is probable that amicronucleate races arise at division by an unequal distribution of the daughter micronuclei.

Some of the literature on amicronucleate ciliates and on the regeneration of various types of nucleate merozoa is reviewed. The evidence shows that the macronucleus is the indispensable nuclear element in the so-called vegetative life of the organism, whereas the micronucleus during this period appears to be a relatively passive organelle. Its chief function concerns the periodic replacement of the macronucleus and the production of new hereditary combinations. Special attention is directed to the fact that inheritance in an amicronucleate race is no less precise than in a typical micronucleate race, although the division of the macronucleus is amitotic and usually reveals no suggestion of true chromosomes. It is evident that the hereditary mechanism of amicronucleate races, and perhaps of ciliates generally, differs radically from the conventional chromosomal mechanism of metazoa.

LITERATURE CITED

BALAMUTH, W., 1940. Regeneration in protozoa: a problem of morphogenesis. *Quart. Rev. Biol.*, **15**: 290–337.

BEERS, C. D., 1944. The maintenance of vitality in pure lines of the ciliate Tillina magna. *Amer. Nat.*, **78**: 68–76.

BEERS, C. D., 1945. Some factors affecting excystment in the ciliate Tillina magna. *Physiol. Zool.*, **18**: 80–99.

BEERS, C. D., 1946. History of the nuclei of Tillina magna during division and encystment. *Jour. Morph.*, **78**: 181–200.

BEERS, C. D., 1946a. Micronucleate and amicronucleate races of Tillina magna. *Anat. Rec.*, **94** (3): 92–93.

BISHOP, E. L., JR., 1943. Studies on the cytology of the hypotrichous infusoria. I. The relation of structure to regeneration. *Jour. Morph.*, **72**: 441–475.

BRESSLAU, E., 1922. Über Protozoen aus Rasenaufgüssen. *Verh. Deutschen Zool. Gesell.*, **27**: 88–90.

BURT, R. L., G. W. KIDDER, AND C. L. CLAFF, 1941. Nuclear reorganization in the family Colpodidae. *Jour. Morph.*, **69**: 537–561.

CALKINS, G. N., 1930. Uroleptus halseyi Calkins. II. The origin and fate of the macronuclear chromatin. *Arch. f. Protistenk.*, **69**: 151–174.

DAWSON, J. A., 1919. An experimental study of an amicronucleate Oxytricha. I. Study of the normal animal, with an account of cannibalism. *Jour. Exp. Zool.*, **29**: 473–513.

DAWSON, J. A., 1920. II. The formation of double animals or "twins." *Ibid.*, **30**: 129–157.

DEMBOWSKA, W. S., 1925. Studien über die Regeneration von Stylonychia mytilus. *Arch. f. Mikr. Anat. u. Entwmech.*, **104**: 185–209.

GREGORY, L. H., 1909. Observations on the life history of Tillina magna. *Jour. Exp. Zool.*, **6**: 383–431.

GRUBER, A., 1879. Neue Infusorien. *Zeit. f. Wiss. Zool.*, **33**: 439–466.

ILOWAISKY, S. A., 1921. Zwei neue Arten und Gattungen von Infusorien aus dem Wolgabassin. *Arb. d. Biol. Wolga-Station*, **6**: 95–106.

KAHL, A., 1931. Die Tierwelt Deutschlands, Part 21, 181–398. Jena: Verlag von Gustav Fischer.

KIMBALL, R. F., 1941. Double animals and amicronucleate animals in Euplotes patella with particular reference to their conjugation. *Jour. Exp. Zool.*, **86**: 1–33.

MAUPAS, E., 1888. Recherches expérimentales sur la multiplication des infusoires ciliés. *Arch. de Zool. Exp. et Gén.*, Sér. 2, **6**: 165–277.

MOODY, J. E., 1912. Observations on the life-history of two rare ciliates, Spathidium spathula and Actinobolus radians. *Jour. Morph.*, **23**: 349–407.

MOORE, E. L., 1924. Regeneration at various phases in the life-history of the infusorians Spathidium spathula and Blepharisma undulans. *Jour. Exp. Zool.*, **39**: 249–316.

PATTEN, M. W., 1921. The life history of an amicronucleate race of Didinium nasutum. *Proc. Soc. Exp. Biol. and Med.*, **18**: 188–189.

PIEKARSKI, G., 1939. Cytologische Untersuchungen an einem normalen und einem Micronucleus-losen Stamm von Colpoda steini Maupas. *Arch. f. Protistenk.*, **92**: 117–130.

POWERS, J. H., AND C. MITCHELL, 1910. A new species of Paramecium (P. multimicronucleata) experimentally determined. *Biol. Bull.*, **19**: 324–332.

REICHENOW, E., 1929. Lehrbuch der Protozoenkunde, Part 1. Jena: Verlag von Gustav Fischer.

REYNOLDS, M. E. C., 1932. Regeneration in an amicronucleate infusorian. *Jour. Exp. Zool.*, **62**: 327–361.

SCHWARTZ, V., 1935. Versuche über Regeneration und Kerndimorphismus bei Stentor coeruleus Ehrbg. *Arch. f. Protistenk.*, **85**: 100–139.

SONNEBORN, T. M., 1940. The relation of macronuclear regeneration in Paramecium aurelia to macronuclear structure, amitosis and genetic determination. *Anat. Rec.*, **78** (Suppl.): 53–54.

TAYLOR, C. V., AND W. P. FARBER, 1924. Fatal effects of the removal of the micronucleus in Euplotes. *Univ. Calif. Publ. Zool.*, **26**: 131–144.

TAYLOR, C. V., AND L. GARNJOBST, 1941. Nuclear reorganization in resting cysts of Colpoda duodenaria. *Jour. Morph.*, **68**: 197–213.

TITTLER, I. A., 1935. Division, encystment and endomixis in Urostyla grandis with an account of an amicronucleate race. *La Cellule*, **44**: 187–218.

TITTLER, I. A., 1938. Regeneration and reorganization in Uroleptus mobilis following injury by induced electric currents. *Biol. Bull.*, **75**: 533–541.

TURNER, J. P., 1937. Studies on the ciliate Tillina canalifera n. sp. *Trans. Amer. Micr. Soc.*, **56**: 447–456.

WENRICH, D. H, 1928. Eight well-defined species of Paramecium. *Trans. Amer. Micr. Soc.*, **47**: 275–282.

WICHTERMAN, R., 1946. Unstable micronuclear behavior in an unusual race of Paramecium. *Anat. Rec.*, **94** (3) : 94.

WOODRUFF, L. L., 1921. Micronucleate and amicronucleate races of infusoria. *Jour. Exp. Zool.*, **34**: 329–337.

WOODRUFF, L. L., 1931. Micronuclear variation in Paramecium bursaria. *Quart. Jour. Micr. Sci.*, **74**: 537–545.

WOODRUFF, L. L., AND H. SPENCER, 1922. Studies on Spathidium spathula. I. The structure and behavior of Spathidium, with special reference to the capture and ingestion of its prey. *Jour. Exp. Zool.*, **35**: 189–205.

YOUNG, D. B., 1922. A contribution to the morphology and physiology of the genus Uronychia. *Jour. Exp. Zool.*, **36**: 353–395.

THE EFFECT OF LOW TEMPERATURE AND OF HYPOTONICITY ON THE MORPHOLOGY OF THE CLEAVAGE FURROW IN ARBACIA EGGS[1]

ALLAN SCOTT

Department of Biology, Union College, Schenectady, N. Y. and The Marine Biological Laboratory, Woods Hole, Massachusetts

When *Arbacia punctulata* eggs are exposed to low temperature during the first cleavage, a pronounced stalk develops between the daughter blastomeres. A stalk also develops at room temperature if the eggs are made to divide in hypotonic sea water or in sea water lacking calcium ion. The development of a conspicuous cleavage stalk is not a normal feature of the first cleavage in Arbacia, although it does occur regularly in some cells; for example, when fibroblasts divide. The object of the work reported here was to examine the conditions under which the stalk is formed in Arbacia and to relate these facts to current theories of the mechanism of cleavage. These particular experimental treatments were used because they were found to affect the appearance of the cleavage stalk.

Methods

Eggs of *Arbacia punctulata* in the first cleavage served as experimental material. Ovulation was induced by the removal of the oral half of the test; eggs emerging from the genital pores were collected in a dish of sea water. The eggs were allowed to settle and the sea water was decanted after which fresh sea water was added. Two such washings were carried out to minimize contamination by coelomic fluid. Fertilization was effected by the use of diluted "dry" sperm, and the sperm were never more than one hour old.

The fertilization membranes were removed by shaking. A heavy suspension of eggs was placed in a five-inch test tube, one-half full of the suspension, and shaken rapidly thirty times. Eggs so treated cleave in time with the controls. The best time for treatment is at 2½ minutes after fertilization, for if shaken earlier, many exovates are formed, and if shaken later, many eggs retain the fertilization membrane. The alternative method of removing the fertilization membrane by treatment with the hatching enzyme (Ishida, 1936) was not attempted.

The hyaline layer was removed in a few experiments by washing the eggs in calcium-free artificial sea water. This was accomplished by several decantations and additions of the calcium-free mixture. It was found that the hyaline layer regenerates somewhat if the eggs are returned to a solution possessing calcium ions; hence if eggs are to lack the hyaline layer, they must be allowed to cleave in the calcium-free mixture.

This study was largely accomplished by photographic means. Photomicro-

[1] This investigation was aided by a Grant-in-Aid from the Sigma Xi Alumni Research Fund.

graphs taken at intervals with Leica-Ibso apparatus, were projected as negatives (1,000 ×) and measurements made with dividers.

Temperature was maintained by means of a thermostatically controlled, water jacketed, glass well, mounted on the microscope stage and connected through a centrifugal pump to a water bath. By this means temperature could be maintained within ± 0.2° C. at or about 20° and within ± 0.4° C. at or about 10° C.

Artificial sea water lacking calcium ions was compounded according to the method of Shapiro (1941). This solution has an osmotic pressure and pH closely similar to that of normal sea water.

The hypotonic solutions were prepared either by the dilution of normal sea water or of the calcium-free mixture.

A few observations are presented on polyspermic eggs cleaving to three or to four cells in one division. Polyspermic development was induced by the method of Smith and Clowes (1924) which involves fertilization in pH 7.2 sea water and the return of the eggs to the normal pH of 8.4 within two or three minutes.

Results

Morphology of the cleavage furrow

The shape of the deepening furrow is markedly different under different conditions; it is influenced by temperature, concentration of calcium ion, tonicity and by presence or absence of the fertilization membrane.

Temperature

At temperatures between 20° C. and 30° C. there is normally no stalk in cleaving eggs whose fertilization membrane has been removed (free cleavage). The furrow is peaked at the apex (Figs. 1 and 2). At low temperatures, 6° to 12° C., a real stalk is formed during the latter part of the furrowing. This occurs whether the egg is enclosed in the fertilization membrane or not. At these low temperatures eggs undergoing membrane-free cleavage, come to resemble a dumbbell with a handle (Figs. 3 and 4).

Calcium ion or urea

Chambers (1938) described the short cleavage stalk which develops when *Arbacia punctulata* eggs divide in calcium-free solutions at room temperature. He used isotonic mixtures of sodium chloride and potassium chloride. In the present study also a short stalk occurred when the eggs were exposed to calcium-free sea water. Similarly a short stalk was figured by Moore (1930a and b) and by Motomura (1934), after treatment with urea solutions.

Fertilization membrane

It is a common practice to remove the fertilization membrane either by dissolving it in urea solutions or by shaking an egg suspension rather violently. These techniques allow the mitotic axis to become much longer and the furrowing is thus more readily followed. If the eggs are confined in the fertilization membrane at 10° C., the blastomeres tend to stay apart and the walls of the furrow are almost vertical (Figs. 11 and 12). At the end of the cleavage a stalk connects the two blastomeres. If this same experiment is varied so that the eggs divide within their

fertilization membranes at 10° C. and in calcium-free sea water, a cleavage stalk likewise develops. In this case, however, the stalk moves eccentrically until it is close to the fertilization membrane (Figs. 30 through 34). The difference is presumably due to the fact that the hyaline layer is dissolved in solutions lacking calcium ion and when the hyaline layer is missing the egg is able to slide around inside the fertilization membrane.

Membrane-free cleavage in polyspermic eggs

Polyspermic eggs may undergo free cleavage to form four cells in the first division. In such cases they frequently divide so that a symmetrical figure is seen from above. In this circumstance the four blastomeres each rest upon the bottom of the glass container (Figs. 15, 16, 17, and 18). Frequently one blastomere rests upon the other three at the end of the cleavage (Fig. 21). The former, more symmetrical type of cleavage is more readily followed. When such an egg begins to cleave it first flattens like a biscuit; at this stage it resembles a balloon around which two rubber bands have been placed at right angles. Such a balloon is flattened on the two surfaces where the rubber bands cross. Perhaps the egg, like the balloon, is subject to greater elastic tension in the region where the incipient furrows cross, and therefore flattens on these surfaces.

As seen from above, the egg periphery is roughly square, with corners rounded (Fig. 15); the wide furrows (at 10° C.) gradually sink towards the center with the apices of the furrows approaching one another. The upper and lower surfaces meanwhile remain relatively flat although the two flat surfaces slowly come together. The whole figure at the stage illustrated in Figure 16 resembles a balloon stretched closely over four tennis balls with two rubber bands placed at right angles. Finally a definitive stalk is formed (Fig. 18).

When the furrows first appear, the egg is to be considered as having two equatorial furrows; that is, two constricting rings (Fig. *29a*), which cross each other. The quasi-independence of the furrows is demonstrated by some eggs which cleave in a similar way but *in which the furrows incise at different rates* (Figs. 19 and 20). In Figure *29b* the furrow separating *ab* from *cd* is well in advance of the furrow separating *ad* from *bc*. This type of cleavage leads to a figure like Figure 20.

It appears that this curious type of cleavage is brought about by the development of two new ring-like tensions which develop around the necks of the individual blastomeres after the deeper furrow is well established. As a result of the deep primary furrow, four new isthmuses are established about the necks of the four incipient blastomeres (*cf.*, Fig. *29b*). Perhaps the most significant feature of

---

PLATE I

FIGURES 1 AND 2. Egg cleaving at 20° C. in sea water, fertilization membrane removed.
FIGURES 3 AND 4. Egg cleaving at 10° C. in sea water, fertilization membrane removed.
FIGURES 5 AND 6. Egg cleaving at 20° C. in 65 per cent sea water, fertilization membrane removed.
FIGURE 7. Egg cleaving within the fertilization membrane at 20° C. in sea water.
FIGURE 8. Late cleavage at 20° C. in 65 per cent sea water, fertilization membrane removed.
FIGURES 9 AND 10. Polyspermic egg fertilized in sea water at pH 7.2, transferred to normal sea water at room temperature until cleavage began. Cleaving at 10° C. in sea water.
FIGURES 11 AND 12. Eggs cleaving within fertilization membrane at 10° C.

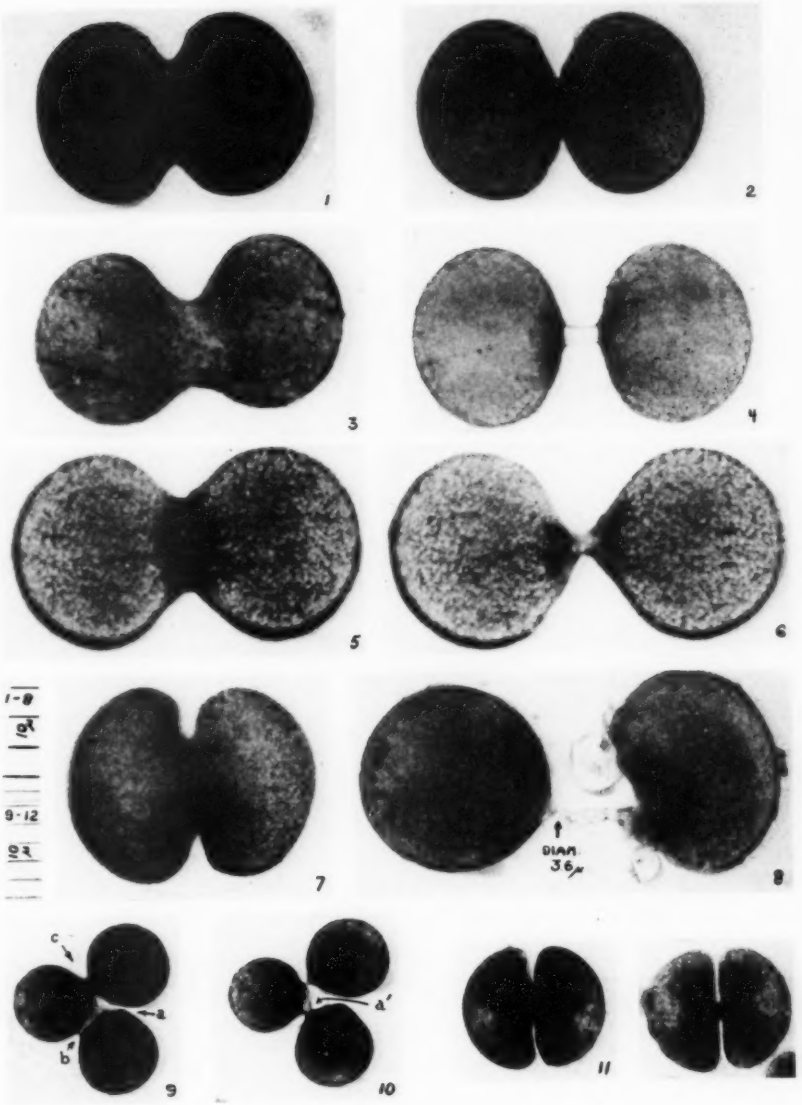


PLATE I

this type of cleavage is the bridge-like stalk which results (Figs. 23 and 24). In these latter figures note that one circumferential furrow deepened symmetrically and more rapidly than the other. The furrow which started later is very asymmetrical, being much deeper on one side (*cf.*, at the arrow) than the other. Eggs cleaving to three cells show a similar behavior (Fig. 22) and when cleavage is com-

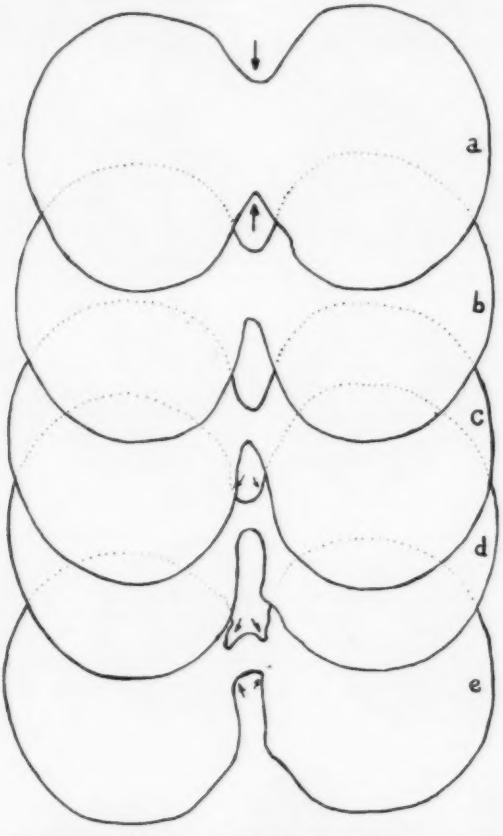


FIGURE 13. Series showing late cleavage and development of the cleavage stalk at 10° C. in sea water.

plete they may have a Y-shaped stalk, or if one furrow deepens more rapidly than the others, two stalks may connect to one blastomere (Figs. 9 and 10).

The speed of furrowing in polyspermic eggs cleaving to four cells may be as rapid as when two cells are being formed, yet it should be remembered that the amount of new surface formed is much greater when a sphere divides into four equal smaller spheres. The surface of a sphere divided into two spheres increases 26 per cent.

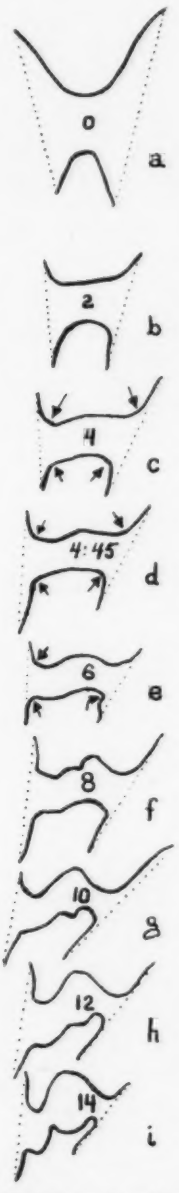


FIGURE 14. Series showing late cleavage and continued activity of cleavage stalk, during fourteen minutes in calcium-free sea water at 11° C.

if divided to four spheres the surface increases 58 per cent. A polyspermic egg cleaving to four cells forms about 26 per cent more surface than the normal first cleavage but it may do so in the same amount of time.

Membrane-free cleavage in hypotonic sea water and in hypotonic calcium-free sea water

Dilution of the sea water causes a swelling of the egg; it also causes an unusually wide furrow to develop during the cleavage and leads to the formation of a stalk at the end (Figs. 5, 6, and 8). This effect occurs at room temperature (20° C.). The stalk may become very long if the sea water has been diluted sufficiently. In mixtures of 65 parts sea water and 35 parts distilled water, for example, the stalk may finally be 30 micra long. This effect occurs either in the presence or absence of calcium ion. The stalk region is certainly a relatively rigid gel, for it has sufficient rigidity to push the daughter blastomeres far apart. Figure 8 and Figures 40 through 42 show the process of elongation in these extreme cases. Enlarged photographs of the stalk at these stages are shown in Figures 43, 44, and 45 with dimensions noted. In Figure 43 the stalk is only 4.4 micra in diameter at one point. In Figure 44 its minimum width is about 2 micra and it is over 22 micra long. In Figure 45 the constriction is completed. The stalk is still 5 micra wide at some points, but it is less than 3 micra in diameter for a third of its length. Chambers (*ibid.*) relates that a spherical oil drop lying within the egg in the furrow region is not deformed until the "external surface of the advancing furrow is 4 to 5 μ from the surface of the oil." If the egg pictured in Figure 43 has a cortex comparable in thickness, then the stalk must certainly be all gel by the time its diameter is reduced to 7 or 8 μ. One blastomere sometimes ruptures when eggs cleave in 65 per cent sea water. No endoplasm escapes if the stalk has closed. One such closed stalk is shown in Figure 28; it is 5 micra in diameter. The conclusion that the stalk is all gel (and yet it continues to constrict) is a most important conclusion for it strongly supports the contraction theory of cleavage of W. H. Lewis. Close inspection at high magnification fails to show any movement of granules located in the stalk. The active constriction of a 5 μ stalk is recorded in Figures 26 and 27.

PLATE II

FIGURES 15, 16, 17, AND 18. Cleavage of a dispermic egg, cleaving in calcium-free sea water at 10° C. Egg fertilized in pH 7.2 sea water, transferred to sea water at room temperature until beginning of cleavage. Time after fertilization: Figure 15, 72 min.; Figure 16, 74 min.; Figure 17, 88 min.; Figure 18, 190 min.

FIGURES 19 AND 20. Egg showing dispermic cleavage. Treatment as in Figures 15–18. Time after fertilization: Figure 19, 86 min.; Figure 20, 88 min.

FIGURE 21. Dispermic egg. Treatment as in Figures 15–18. One blastomere out of the horizontal plane.

FIGURE 22. Diagram illustrating two types of cleavage to three cells.

FIGURES 23 AND 24. Egg in 70 per cent sea water at 25° C., after accidental polyspermy. Time after fertilization: Figure 23, 50 min.; Figure 24, 52 min.

FIGURE 25. Dispermic egg cleaving in sea water at 11° C., following fertilization in pH 7.2 sea water.

FIGURES 26 AND 27. Late cleavage of egg in 65 per cent sea water. Room temperature. Time after fertilization: Figure 26, 83 min.; Figure 27, 85 min.

FIGURE 28. Closed stalk following rupture of one blastomere; 65 per cent calcium-free sea water.

FIGURE 29. See text.

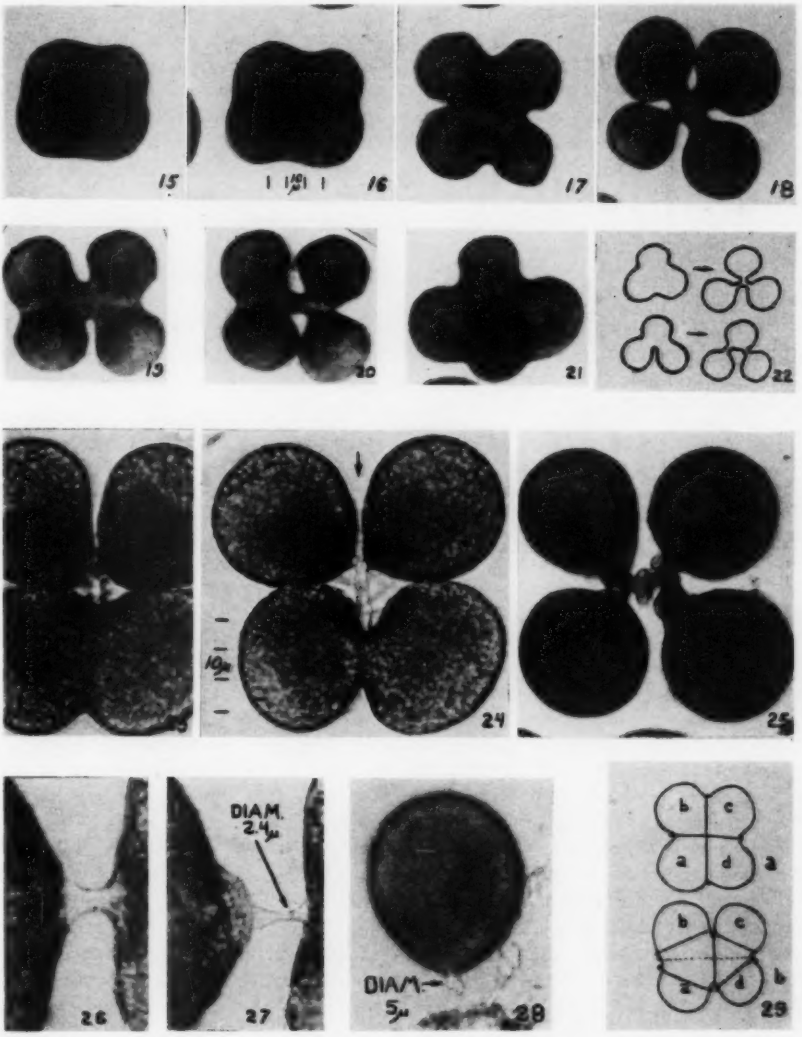


PLATE II

The stalk

The mitotic axis (greatest length) of eggs undergoing free cleavage becomes progressively longer at 10° than at 20° C. (compare Figs. 1 and 2 with 3 and 4); moreover the early furrow at 10° C. is much more blunt in contour than is the furrow of eggs at higher temperatures. A study of the final phase of cleavage under high power (Fig. 13) shows how the wide furrow is transformed into a stalk.

In Figure 13*a* the deepening furrow is still blunt with a diameter of about 14 micra. In Figure 13*b*, however, the stalk is beginning to square off. The arrows (Figs. 13*d* and *c*) indicate the region where the constriction is most active. The details are similar and are very clear in eggs cleaving in calcium-free sea water at 10° C. The series of diagrams shown in Figure 14, *a* to *i*, again show that the broad furrow first deepens until the diameter of the waist is about 7 or 8 micra (*a* and *b*), then the stalk is elongated by the constriction of the subequatorial cortex (*c* and *d*, see arrows); meanwhile the entire stalk is diminishing in diameter. The minimum diameter of the stalk is about 4 micra at 10° C. and in calcium-free sea water; in hypotonic solutions the diameter is often less. When the diameter of the stalk diminishes below 4 micra, it does so in local areas only (*cf.*, Fig. 14*g*, *h*, *i*).

Amoeboid activity and cleavage activity

Many workers have noted that the polar surface of the cell bubbles actively during cytokinesis (Bowen, 1920—in *Euchistus spermatocytes;* and Lewis 1942—in tissue culture fibroblasts). This is not the case with the egg of the sea urchin during the first cleavage, instead the polar surface remains smooth and inactive. However, a variety of agents, will cause the formation of blebs in the sub-furrow region. One such agent is hypotonic calcium-free sea water. The blebs usually begin to form after the completion of the major furrowing and they give rise to sizable spherules which are cut off by a process very much like cleavage (Figs. 46*a*, *b*, *c*). The inactivity of the polar surface may indicate that the cortex there is different from the equatorial cortex in Arbacia.[2]

Eggs that have been in the hypotonic medium for some time may show a sudden rush of endoplasm from one blastomere to the other, often causing the blastomeres to become very unequal in size (Fig. 47 and Figs. 35 to 39).[3] This endoplasmic flow is a very rapid one, usually lasting only two or three seconds. It is remarkable, however, that the flow is accompanied by a rapid deepening of the furrow, appearing as though a tension has been suddenly overcome, allowing the furrow to constrict much more rapidly than usual. The sub-cortical flow in such eggs may be down one side of the furrow, through the constricted stalk and up the other side of the furrow, yet the furrowing continues normally to completion (Fig. 47).

[2] The view that there is a special substance (a special type of plasmagel) located around the equator has been espoused by a number of workers, Marsland (1942) and Lewis (1942) among others. Beams and King (1937) are of the opinion that they have removed the "surface active material" of Ascaris eggs by centrifugation at 150,000 × gravity.

[3] The rush of endoplasm described is in this case related to cleavage. It resembles the amoeboid activity described by Moser (1940) after urea treatment. Moser, p. 77, cites other cases from the literature.

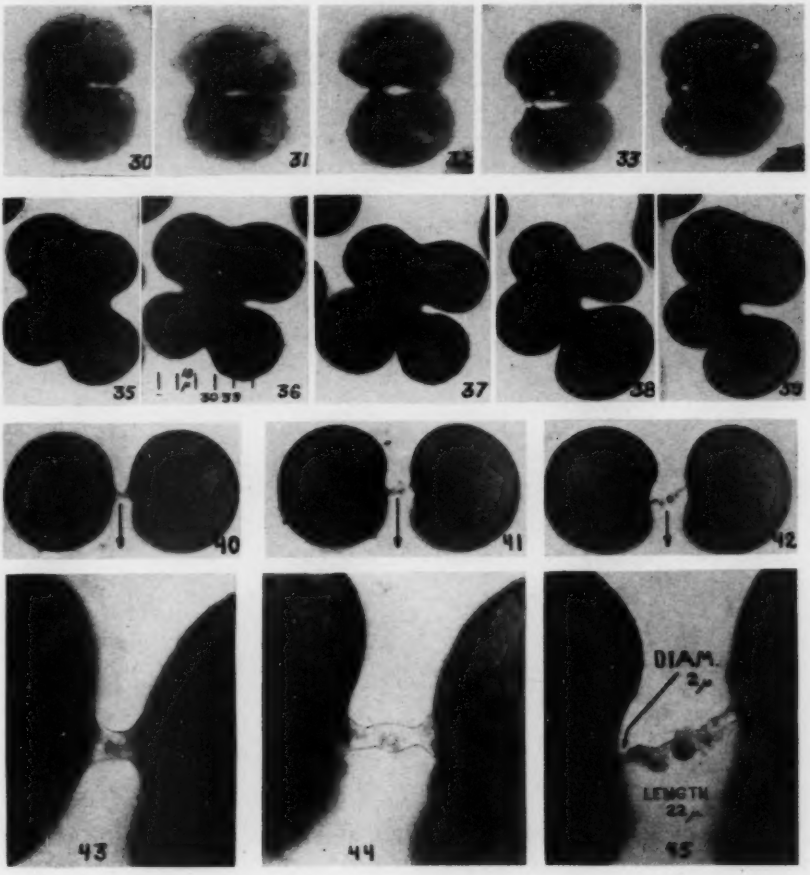


PLATE III

FIGURES 30, 31, 32, 33, AND 34. Eccentrically placed cleavage stalk. Eggs in fertilization membrane at 10° C., in calcium-free sea water.

FIGURES 35, 36, 37, 38, AND 39. Volume changes of individual blastomeres. Calcium-free sea water.

FIGURES 40, 41, AND 42. Elongation of the cleavage stalk; in 65 per cent sea water at room temperature. Time intervals: Figures 40–41, 1 min. and 40 sec.; Figures 41–42, 1 min. and 35 sec.

FIGURES 43, 44, AND 45. Enlargements of Figures 40, 41, and 42. The edges of the nearly transparent stalk have been retouched in Figures 4, 8, 26, 27, 28, 43, 44, and 45.

Discussion

The stalk

The occurrence of a stalk during the cleavage of the Arbacia egg is correlated with the degree of gelation of the furrow cortex. Both the observations made in this paper and those of other workers who have concerned themselves with the degree of gelation of the egg cortex confirm this. The results of several workers are summarized below:

Brown (1934): Cortical pigment granules are especially resistant to displacement by centrifugation during the division phase.

Chambers (1938): The furrow cortex resists disintegration after the two incipient blastomeres have been punctured at the poles.

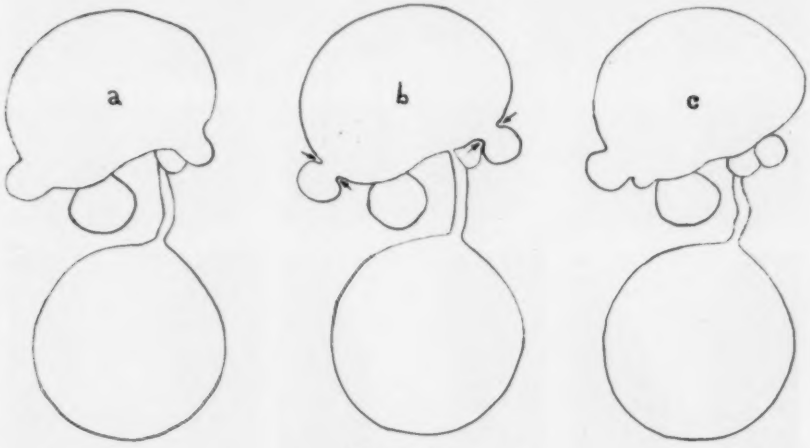


Figure 46

Brown and Marsland (1936): There is a quantitative decrease in the gel value of dividing eggs as the hydrostatic pressure is increased. Under high pressures the furrow regresses.

No one has yet recorded the effect of temperature, hypotonicity and lack of calcium ion upon the cortex of the dividing Arbacia egg, although these observations have been made upon the unfertilized egg. A brief summary of this work follows:

Costello (1938): It takes progressively longer to fragment the eggs as the temperature is lowered.

Cole (1932) and Harvey (1943): It takes longer to fragment eggs in hypotonic than in isotonic solutions.

Harvey (1945): Arbacia eggs break less readily in solutions possessing calcium ions than in solutions lacking calcium ions.

These treatments (low temperature, hypotonicity and calcium ion) are precisely the ones which favor the development of a cleavage stalk. It is possible that

these treatments may increase the elastic strength of the egg surface by toughening the extra cortical structures, but it is probable that they favor cortical gelation as well.

Hypotheses concerning the mechanism of cleavage; surface tension

Chambers and Kopac (1937) found that clean oil drops of the proper interfacial tension with sea water, will coalesce spontaneously with a naked egg (Arbacia, Lytechinus, and Echinometra). They state: "The tendency to coalescence in the furrow and polar zones of cleaving eggs (late amphiaster and later) was investigated and no difference was found." They used oils whose approximate tensions in contact with sea water were 30, 10, and 3 dynes per cm. The fact that coalescence occurs at all indicates a fluid layer around the egg periphery. Spontaneous coalescence does not occur in *Amoeba proteus* (Kopac and Chambers, 1937), which indicates a non-fluid surface. In view of these observations any surface tension hypothesis is untenable.

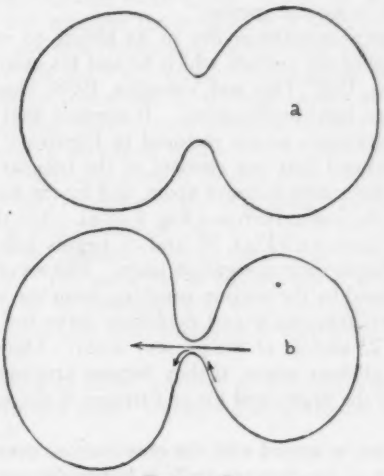


FIGURE 47

Subcortical currents

Chambers (1938) has hypothesized that cleavage results from the activity of "the sub-cortical currents (which) sweep around the two asters and add gelating material to the inwardly growing cortex." In this hypothesis he combines his own observations with those of Schechtman (1937) on localized cortical growth during the cleavage of the amphibian egg. It was shown in the present paper (page 280) that normal furrowing may be associated with abnormal currents, which argues against the importance of such currents for division; moreover Lewis (1942) found no currents in the dividing fibroblast.

Astral cleavage

An astral theory of cleavage, much modified from Gray (1931), has been elaborated by Katsuma Dan (1943). He believes that the asters are composed of radiate fibers with intrinsic rigidity; he considers them to be anchored to the cortex; he believes that the rays cross at the equator; and he believes that the spindle elongates autonomously. The following quotation (Dan 1943) summarizes his theory of cytokinesis: ". . . it was also shown that this concept of the mechanism of cell division is adequate to explain the stretching phase of the furrow surface. That is, when two such radiate asters are pushed apart, they can in turn, push the cell membrane of the polar region somewhat as a paint brush would push some object. As they travel away, however, since they enclose the fluid endoplasm within the interspaces of their rays, the fluid endoplasm is carried away from the equatorial region and the cortex there is sucked in, giving rise to a furrow. The cortex is stretched as it is pulled in by the suction."

The strength of Dan's hypothesis lies in its ability to explain the differential stretching and shrinkage of the surface which he and his coworkers observed (Dan, Yanagita, and Sugiyama, 1937; Dan and Yanagita, 1938; Dan, 1943) and for which no other explanation has been forthcoming. It appears that Dan's hypothesis will explain such unusual cleavages as are pictured in Figures 9 and 10 of the present paper. It could be assumed that one element of the tripolar spindle elongated before the others causing the asters to move apart, and by the suction mechanism, causing the development of the initial furrow (Fig. 9 at *a*). On this assumption the development of the other furrows (Figs. 9*b* and *c*) begins later, presumably because the other two spindles begin their elongation later. The secondary furrow (Fig. 10 at *a'*) is presumably caused by the suction resulting from the separation of the lower two asters. Similar explanations would doubtless serve for the tetra-astral cleavages shown in Figures 23 and 24 of the present study. One can imagine also that the crossing rays from all four asters, if they became attached to the cortex, would explain the flattening of the upper and lower surfaces of the egg observed in Figure 15.

Dan's hypothesis is not in accord with the observations presented here concerning the continued elongation of the cleavage stalk in hypotonic sea water for it is impossible to see how the astral suction mechanism could explain the further constriction of a long, completely gelated stalk.

The main weakness of the astral suction hypothesis lies in its limited scope. It fails to explain undoubted cases of anastral cleavage (tissue culture, for example) frequently noted in the literature. Dan's easy conclusion that all of these anastral cases are explainable by his astral suction hypothesis (". . . it is possible to imagine that in cells of the anastral type, similar gelation systems may be existing although they cannot be discerned morphologically") is unconvincing.

One of Wilson's observations is discordant with Dan's hypothesis. Wilson observed, in a form which normally has asters, that a spindle need not be present for complete cleavage to occur. He found that a cleavage furrow may cut in around the base of an isolated aster and result in a complete cleavage. Compare Wilson (1901), page 376 and Figure 11.

In one of Chamber's microdissection experiments he bisected the partially cleaved egg in a plane at 45° to the plane of the furrow (1924, Fig. 36). The cut resulted

immediately in two cells. However, the original furrow remained on each artificially produced blastomere and, on each, the furrow gradually cut through forming two small "cells" as well as two large ones. This continued cleavage seems to be quite unexplainable by Dan's hypothesis which requires crossed astral rays, an elongating spindle and a suction produced by the separation of the asters.

Cortical growth or cortical contraction?

Schechtman has proposed another theory of the mechanism of cytokinesis. He suggested (1937) that the furrow cortex grows by the "intussusception of clear cytoplasm," but simple growth of the equatorial cortex would not be expected to cut the egg in half. Other factors must account for the inwardly directed furrow and its narrowing. It seems clear that there is a stretching of the egg cortex at the time of furrowing as concluded by Dan et al. (1937, 1938), by Schechtman (1937) and by Motomura (1940), but whether the stretching is active (the result of growth) or whether it is passive and due rather to a contracting ring at the head of the furrow (Lewis, 1942), is not easy to decide. Schechtman is of the opinion that "Cleavage is initiated by a contraction of the egg cortex at the site of the future furrow." And he notes that the "Cortex becomes thicker and bulges toward the egg interior." He therefore uses both contraction and cortical growth in his complete hypothesis. The observations made in this paper on the continued constriction of small stalks after they consist entirely of gelated material are taken as strongly favoring the constricting ring theory of Lewis. For if the gelated stalk is able to contract at that late stage of cleavage, it seems reasonable to suppose that it possesses contractile power earlier. The direction of contraction is ringwise about the equator (Fig. 29*a*) and it is to be expected that such contraction would draw stained areas out into fine lines as Schechtman observed, if such areas are located in the furrow or subfurrow region.

It would be illuminating to know whether or not kaolin particles placed *around* the equator would be brought closer together during the furrowing but no one has made these observations.

Amoeboid activity and bleb formation

One can scarcely observe the amoeboid behavior of eggs in hypotonic media and particularly the "normal" false cleavages which occur during the amoeboid phase preceding pronuclear fusion in the nematode egg (Spek, 1918), without being convinced that a fundamental similarity exists between amoeboid motion and cleavage. Moreover the abscission of blebs is strikingly similar to cleavage.[4] It is suggested that any deforming force which establishes an isthmus about the cell or a part of the cell will result in the development of a contracting ring disposed around the isthmus, provided that the egg is in the cleavage phase. This view would explain why the normal egg, deformed by the elongating spindle, cleaves at the equator. It would explain why cleavage planes cut in around the base of cytasters which are unconnected to a spindle (Wilson, 1901) and it would explain why blebs formed in

[4] Very recently Holtfreuter (1946) has suggested "that in normal cytoplasmic division the activity of the nucleus and of the endoplasm are of a mere secondary importance." He observed that isolated, embryonic amphibian cells may develop annular constrictions which lead to the fragmentation of the cell. He considers, however, that the contraction occurs in the membrane rather than in the plasmagel layer.

the sub-furrow region may cut off from the remainder of the egg as reported above. This hypothesis also agrees with the idea that the enlarging gelated asters play a mechanical role in localizing the furrow.

Summary

1. Under certain conditions the eggs of *Arbacia punctulata* develop a cleavage stalk between the first two blastomeres. No stalk forms in sea water if the temperature is in the 20° C. to 30° C. range; low temperature (10° C.) causes the development of a stalk in sea water; a short stalk develops in isotonic calcium-free sea water at 20° C.; a very long stalk develops if eggs are cleaving in hypotonic sea water (65 per cent).

2. The effect of the above treatments on the appearance of cleaving dispermic eggs is described.

3. Evidence indicates that stalks of 8 micra diameter are all gel, yet in hypotonic sea water they continue to constrict and elongate. This is good evidence that the cortical gel has inherent contractile properties.

4. It is hypothesized that any event which deforms the Arbacia egg (if it is in the "cleavage phase") leads in some way to an orientation of contraction around the isthmus. The deforming force may be an enlarging aster, an elongating spindle, or an endoplasmic flow.

LITERATURE CITED

Beams, H. W. and R. L. King, 1937. The suppression of cleavage in Ascaris eggs by ultracentrifuging. *Biol. Bull.*, **73**: 99–111.

Bowen, R. H., 1920. Studies on insect spermatogenesis. I. *Biol. Bull.*, **39**: 316–362.

Brown, D. S., 1934. The pressure coefficient of "viscosity" in eggs of Arbacia punctulata. *Jour. Cell. Comp. Physiol.*, **5**: 335–346.

Brown, D. S. and D. A. Marsland, 1936. The viscosity of Amoeba at high hydrostatic pressure. *Jour. Cell. Comp. Physiol.*, **8**: 159–165.

Chambers, R., 1924. The physical structure of protoplasm as determined by micro-dissection and injection. *General cytology*, pp. 237–309. The University of Chicago Press.

Chambers, R., 1938. Structural and kinetic aspects of cell division. *Jour. Cell Comp. Physiol.*, **12**: 149–165.

Chambers, R. and M. S. Kopac, 1937. The coalescence of sea urchin eggs with oil drops. *Ann. Report Tortugas Laboratory, Carnegie Inst. of Washington.* No. 36: 88.

Cole, K. S., 1932. Surface forces on the Arbacia egg. *Jour. Cell. Comp. Physiol.*, **1**: 1–9.

Costello, D., 1938. The effect of temperature on the rate of fragmentation of Arbacia eggs subjected to centrifugal force. *Jour. Cell. Comp. Physiol.*, **11**: 301–307.

Dan, K., 1943. Behavior of the cell surface during cleavage. VI. On the mechanism of cell division. *J. Fac. Sci., Tokyo Imp. Univ.*, Sec. IV, **6**: 323–368.

Dan, K., J. C. Dan and T. Yanagita, 1938. Behavior of the cell surface during cleavage. II. *Cytologia*, **8**: 521–531.

Dan, K., T. Yanagita and M. Sugiyama, 1937. Behavior of the cell surface during cleavage. I. *Protopl.*, **28**: 66–81.

Gray, J., 1931. *A text book of experimental cytology.* Cambridge University Press.

Harvey, E. B., 1943. Rate of breaking and size of "halves" of the Arbacia punctulata egg when centrifuged in hypo- and hyper-tonic sea water. *Biol. Bull.*, **85**: 141–150.

Harvey, E. B., 1945. Stratification and breaking of the Arbacia punctulata egg when centrifuged in single salt solutions. *Biol. Bull.*, **89**: 72–75.

Holtfreuter, Johannes, 1946. Structure motility and locomotion in isolated embryonic amphibian cells. *Jour. Morph.*, **79**: 27–62.

Ishida, Juro, 1936. An enzyme dissolving the fertilization membrane in sea urchin eggs. *Annotationes Zoologicae Japonenses*, **15**: 449–459.

KOPAC, M. S. AND R. CHAMBERS, 1937. The coalescence of living cells with oil drops. *Jour. Cell. Comp. Physiol.*, **9**: 345–361.

LEWIS, W. H., 1942. The relation of the viscosity changes of protoplasm to amoeboid motion and cell division. *The structure of protoplasm*, pp. 163–197. Iowa State College Press.

MARSLAND, D., 1942. Protoplasmic streaming in relation to gel structure in the cytoplasm. *The structure of protoplasm*, pp. 127–161. Iowa State College Press.

MOORE, A. R., 1930a. Fertilization and development without membrane formation in the egg of the sea urchin Strongylocentrotus purpuratus. *Protopl.*, **9**: 9–17.

MOORE, A. R., 1930b. Fertilization and development without membrane formation in the egg of Dendraster eccentricut. *Protopl.*, **9**: 18–24.

MOSER, F., 1940. Studies on a cortical layer response to stimulating agents in the Arbacia egg. III. *Biol. Bull.*, **78**: 68–91.

MOTOMURA, I., 1934. On the mechanism of fertilization and development without membrane formation in the sea urchin egg, with notes on a new method of artificial parthenogenesis. *Science Reports of the Tohoku Imp. Univ., 4th series, Biol.*, **9**: 33–45.

MOTOMURA, I., 1940. Studies of cleavage. I. *Science Reports of the Tohoku Imperial Univ., 4th series, Biol.*, **15**: 123–130.

SCHECHTMAN, A., 1937. Localized cortical growth as the immediate cause of cell division. *Science*, **85**: 222–223.

SHAPIRO, H. H., 1941. Centrifugal elongation of the cell and some conditions governing return to sphericity, and cleavage time. *Jour. Cell. Comp. Physiol.*, **18**: 61–78.

SMITH, H. W., AND G. H. A. CLOWES, 1924. The influence of hydrogen ion concentration on the fertilization process in Arbacia, Asterias and Chaetopterus eggs. *Biol. Bull.*, **47**: 333–344.

SPEK, J., 1918. Die amöboiden Bewegung und Strömungen in die Eizellen einiger Nematoden während der Vereinigung der Vorkerne. *Arch. f. Entw. Mech.*, **44**: 217–254.

WILSON, E. B., 1901. Experimental studies in cytology II and III. *Arch. f. Entw. Mech.*, **13**: 353–395.

DEVELOPMENTAL RELATIONS BETWEEN GENITAL DUCTS AND GONADS IN DROSOPHILA

DIETRICH BODENSTEIN

Medical Division, Edgewood Arsenal, Maryland

While studying the reproductive system of *Drosophila simulans* gynandromorphs Dobzhansky (1931) made the following interesting observation: If female genital ducts and testes were present in the same individual and if the female ducts were attached to the testes, the latter underwent extreme degeneration. Yet, the attachment of male genital ducts to ovaries did not affect the development of these organs.

Now we know that in normal development the attachment of the ducts to the gonads takes place during the early period of pupal life. We know further that by transplantation of gonads from one individual to another it is possible to obtain attachment of the transplanted organ to the host ducts. This knowledge makes it possible to attack experimentally the question whether the degeneration of testes when attached to the female ducts, as observed in *Drosophila simulans* gynandromorphs, is a peculiarity of this special case, or whether the phenomenon is a general one and always occurs when female ducts and testes are brought into contact with each other.

The problem can be approached experimentally in two ways: the larval testes can be transplanted into female host larvae or the female genital disc from which the ducts originate can be transplanted into male larvae. By transplanting two or three testes into one host, the chance that one transplantal will attach itself to the host duct is quite good. The chances for attachment of the testes to the female duct are even better when the six oviducts which arise by outgrowths from the three transplanted imaginal discs compete with the two host ducts for attachment. In the following studies both these methods were used.

Experimental

Transplantation of testes into female ducts

Two or three testes of mature virilis larvae were transplanted together into the abdominal cavity of female host larvae of the same age. After the hosts had emerged, the condition of the transplants and their relationship to the female genital system was studied in careful dissections. This series consisted of ten cases. The following was found. All transplants had failed to assume their characteristic spiral shape. This was to be expected, since the work of Dobzhansky (1931) and Stern (1941a and b) had shown that the testes have to be attached to the vas in order to accomplish their spiral growth. In six out of ten cases one of the transplanted testes had attached itself to one of the oviducts of the hosts. The attached testis was always greatly reduced in size and appeared degenerate. Figure 1 shows camera lucida drawings of five representative cases of this series. It will be noted that the degenerative reduction occurs only when the testis is attached to the *oviduct* of

the host (Fig. 1*A*, *B*, *D* and *E*). Testes that lie free in the body cavity (Fig. 1*A* and *B*) or testes that have been attached to the ovary (Fig. 1*C* and *B*) are unaffected. Thus the principle which produces degeneration is apparently given off only by the oviducts and depends for its action on a close cellular contact with the testes. This principle, moreover, seems unable to penetrate larger cell barriers, for testes which were connected to ovaries which in turn had their normal oviduct connection remained unaffected (Fig. 1*C*). Yet two testes which had established close

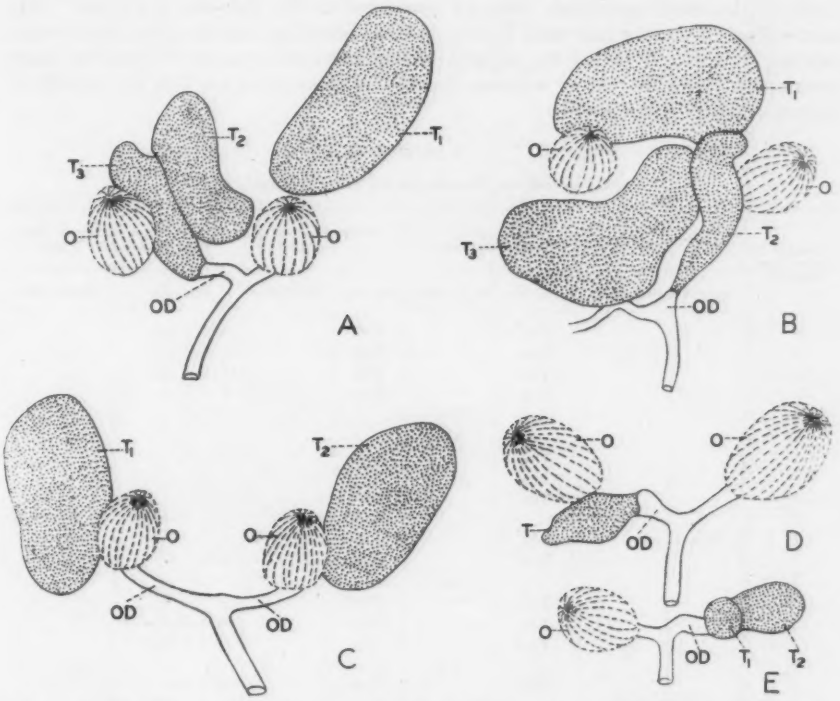


FIGURE 1. The spatial and developmental relations of transplanted testes to the reproductive system of their female hosts. *O*, ovary; *OD*, oviduct; T_1 to T_3, transplanted testes.

contact with each other had both suffered degenerative reduction, although only one of these fused organs has actually established contact with the oviduct (Fig. 1*A* and *E*).

Transplantation of female genital discs into male hosts

In a second series of experiments, two or three female genital discs from mature virilis larvae were transplanted together into the body cavity of hosts of the same age. The condition of the host testes and their relationship to the transplanted female structures was again studied by dissection. Several of the affected testes

were also sectioned and studied histologically. Thirty successful cases were available for investigation. In seven of these cases, the host testes were not connected to the transplanted ducts, although the latter had developed well and were found in the immediate neighborhood of the male gonads. The testes of these seven hosts were normal in size, shape, and histology. One testis in each additional animal was not connected with the vas of the host, nor to any of the transplanted ducts. These testes were not coiled but were otherwise normal. The other testis in two of these individuals was connected to the vas of the host and was normal, while the other testis of the third individual, although connected to the vas, was not coiled. The non-coiling of an attached testis is rare, but has been observed at times in otherwise normal animals. Whether the inability of attached testes to coil is a result of faulty connections with the vas or whether the vas in these cases has lost its growth inducing capacity is not known.

TABLE I

Transplantation of female genital ducts into male hosts

Number of discs transplanted	Testis free (round)		Testis (spiral). Normal attached		Testis attached to ♀ duct		State of degeneration of testis when attached to ♀ duct	
	One side	Both sides	One side	Both sides	One side	Both sides	One side	Other side
3			Yes*		Yes		+	
3			Yes		Yes		++++	
3			Yes		Yes		++++	
3			Yes		Yes		++++	
3				Yes				
3				Yes				
3			Yes		Yes		++++	
2			Yes		Yes		+++	
2			Yes		Yes		++++	
2						Yes	+++++	+++++
2	Yes		Yes				+	
3			Yes		Yes		++	
3				Yes				
3			Yes		Yes		++++	
3			Yes		Yes		+++++	
3			Yes		Yes		+++++	
2				Yes				
2			Yes		Yes		+++	
2		Yes						
2		Yes						
2		Yes						
2				Yes				
2				Yes				
2						Yes	+++++	+++++
2			Yes		Yes		++++	
2						Yes	+++	+++++
2 h.				Yes				
2 h.			Yes		Yes		++++	
2 h.			Yes		Yes		++++	
2 hv.			Yes		Yes		++++	

h. = hydei discs into hydei hosts. hv. = hydei discs into virilis host. * = testis attached to vas, but not spiral.

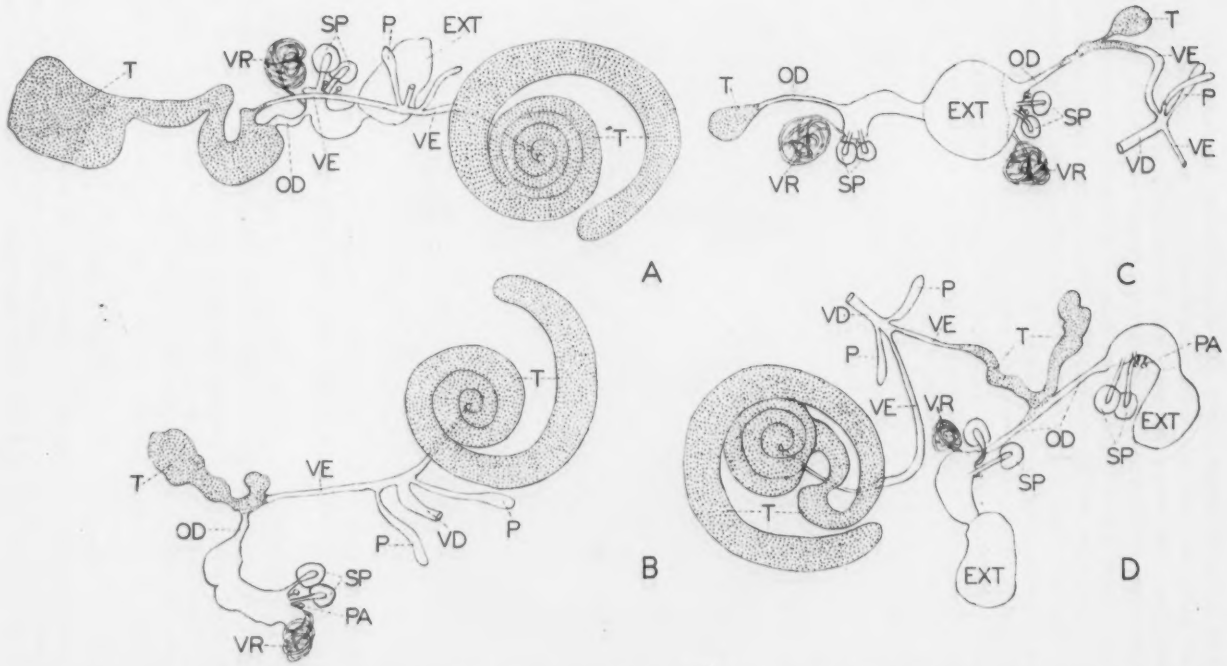


FIGURE 2. The spatial and developmental relations of transplanted female genital structures to the reproductive system of their male hosts. *EXT*, external female genitalia formed by the transplants; *OD*, oviduct; *P*, paragonium; *PA*, parovaria; *SP*, *spermathecae*; *T*, testis; *VD*, vas deferens; *VE*, vas efferens; *VR*, ventral receptacle.

In twenty individuals one or both testes were connected to the oviduct of the transplant. In some cases one testis was even found to have connection with the oviducts of two discs. All testes that were attached to oviducts showed a more or less pronounced degree of reduction and appeared degenerate. Table I summarizes the results of this experimental series. The state of degenerative reduction of the testes in this table is indicated by crosses. One cross signifies slight; six crosses, extreme size reduction. Figure 2 shows camera lucida drawings of four representative cases of this series. Figure 2*A* is a case listed in Table I having one cross. Figure 2*C* is listed by having six crosses and Figure 2*D* and *B* are listed by having five crosses each. Figure 3 illustrates by microphotography an extremely reduced testis.

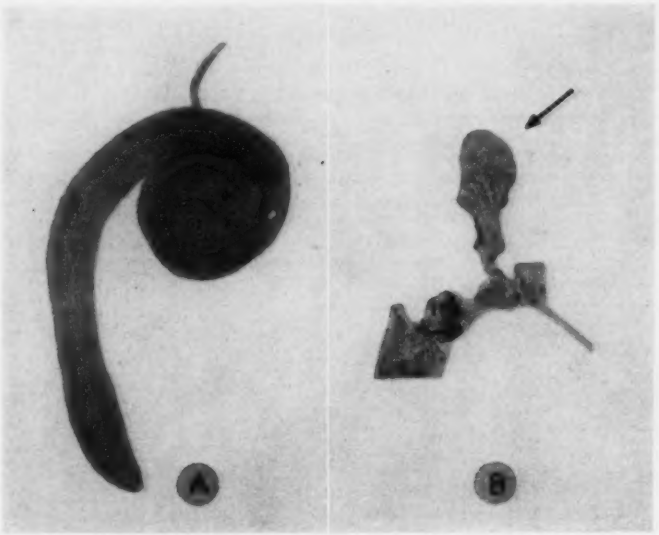


FIGURE 3. *A*, normal adult testes. *B*, (arrow) testis of the same individual degenerated under the influence of a transplanted and attached oviduct.

It will be noted from Table I that the reduction of the testes is in most cases very pronounced. Only six of the testes attached to oviducts were reduced to state "3" or less, while sixteen testes were degenerated to state "4" or more.

This experimental series thus confirms strikingly the original observation that testes attached to female oviducts suffer degenerative changes and that it is the oviduct that elicits the principle causing degeneration. The observation from the previous experiments that the presence of oviducts *per se* has no effect on testis development is also confirmed, for testes in the presence of as many as three pairs of oviducts in the immediate organic environment remained normal if they were not attached to the oviduct. In comparing the attached testes of the two series with each other, a difference in their general shape was noted. While the transplanted

testes in the first experimental group were small, roundish bodies, the testes in the second experimental group were in most cases thin and elongated in shape (compare Fig. 1 with Fig. 2). Now it was found in the second group that in all cases when the attached testis was thin and elongated it was attached not only to the transplanted oviduct but also to the vas efferens of its host (Fig. 2). In those cases, however, where the testis attached only to the transplanted oviduct, had not established connection with the vas, it was roundish. This situation is well illustrated in Figure 2*C*. The left testis in this case, a small roundish degenerated organ, is attached only to the oviduct while the right testis, which is attached to the vas efferens of the host and to one transplanted oviduct, has become an irregularly elongated structure. The elongated shape of such a degenerate testis is thus due to the stimulating influence of the vas on the growth of the testis, which in normal development leads to the coiling of this organ, while the observed degeneration is caused by the influence of the oviduct.

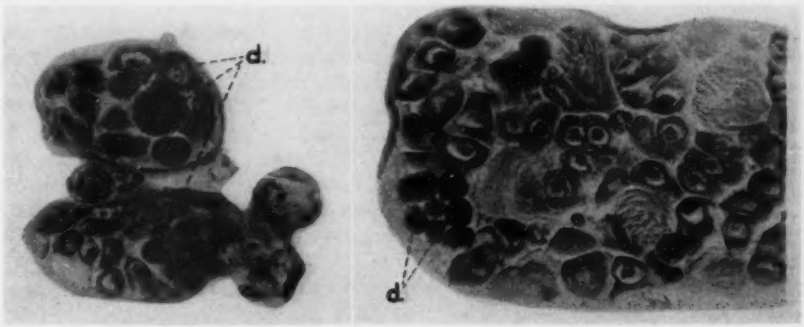


FIGURE 4. Sections through two extremely reduced testes. *d*, degenerating cells.

Not only virilis but also hydei oviducts cause degeneration of hydei testes attached to them. This was shown by three cases in which larval hydei female discs were transplanted into hydei male larvae (see Table I).

The factor in the oviduct causing degeneration of the testis by contact is not species specific, for hydei oviducts will cause virilis testes to degenerate (see Table I).

Sections of reduced testes were made and their histology studied. It was found that, depending upon the degree of reduction of size, the testes contained various amounts of spermatogonia and spermatocytes in all stages of degeneration. The remnants of disintegrated cells in the form of granular picnotic masses together with quite normal appearing cells were observed. Figure 4 shows the condition found in an extremely reduced testis.

CONCLUSION AND SUMMARY

By transplanting female genital discs into male hosts, attachment to the host testes of oviducts developed from transplanted genital discs is obtained. In these cases the attached testes suffer extensive degeneration. Only cellular contact of

the oviducts to the testes brings about this phenomenon. Unattached female ducts do not affect the development of the testis. The principle causing degeneration is not species specific. The findings indicate that the phenomenon encountered is no unique instance, but representative when oviduct and testis establish cellular contact during pupal development.

LITERATURE CITED

BODENSTEIN, D., 1946. The post-embryonic development of Drosophila. In Biology of Drosophila. *Publ. Carnegie Inst. Wash.* (in press).

DOBZHANSKY, T., 1931. Interaction between female and male parts in gynandromorphs of Drosophila simulans. *Roux' Arch. Entw. Mech.*, **123**: 719–746.

STERN, C., 1941a. The growth of testes in Drosophila. I. The relation between vas deferens and testis within various species. *Jour. Exp. Zool.*, **87**: 113–158.

STERN, C., 1941b. The growth of testes in Drosophila. II. The nature of interspecific-differences. *Jour. Exp. Zool.*, **87**: 159–180.

A HISTOLOGICAL STUDY OF SYNDISYRINX FRANCISCANUS, GEN. ET SP. NOV., AN ENDOPARASITIC RHABDOCOEL OF THE SEA URCHIN, STRONGYLOCENTROTUS FRANCISCANUS[1]

H. E. LEHMAN

Department of Zoology of the University of North Carolina[2]

Introduction

Up to the present time eight genera of worms endoparasitic in echinoderms and sipunculids have been described that belong to the rhabdocoel family Umagillidae Wahl, 1910b. Schneider described the first species, *Anoplodium parasita*, in 1858. Since then six questionable and three valid species of this genus have been reported from widely separated localities as parasites of holothurians. Their distribution extends from the Mediterranean, Ionian, and North Seas to Japan and the Philippines (Bock, 1926). *Syndesmis echinorum* Francois, 1886, the only species of the genus, is found in echinoids. It has been collected in the Mediterranean (Russo, 1895), Norway (Westblad, 1926), and the English Channel (Braun, 1889). Three species of the genus *Collastoma* are found in sipunculids at Roscoff (Dörler, 1900), the Gulf of Kola (Beklemischev, 1916), and the Bay of Naples (Wahl, 1910a). The genus *Desmote* is represented by one species, *D. vorax*, discovered in a crinoid collected in the Gulf of Kola (Beklemischev, 1916). A single species parasitic in holothurians has been described in each of four genera, i.e., a Japanese form, *Xenometra arbora* Ozaki, 1932, and three reported from the coast of Norway, *Wahlia macrostylifera* Westblad, 1930, *Anoplodiera voluta* Westblad, 1930, and type genus *Umagilla forskalensis* Wahl, 1909.

The only reference to a member of the Umagillidae from the Western Hemisphere was made by Powers in 1936. He reported the presence of a Syndesmis-like worm in the coelomic cavity of the echinoid, *Centrechinus antillarum*, at Tortugas. A complete description was not given; however, as compared with *Syndesmis*, noticeable differences were observed in details of the copulatory apparatus and the arrangement of the shell glands. While the endoparasitic rhabdocoel of *Strongylocentrotus franciscanus*, the large common sea urchin of the California coast, is well known to some investigators who have worked at Pacific Grove, a description of this worm has not been recorded in the literature prior to the present account.

[1] This work was done at the Wilson Zoological Laboratory of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts. The author is indebted to Professor D. P. Costello for suggesting the problem, for the slide preparations upon which this study was essentially based, and for the invaluable suggestions and criticisms rendered during the preparation of this paper. The author wishes to acknowledge his appreciation to Dr. L. H. Hyman for many valuable recommendations and for permission to introduce her revised and hitherto unpublished terminology relating to this group. To Miss Catherine Henley the author expresses his gratitude for the translation of a number of the references cited herein.

[2] Now in the School of Biological Sciences of Stanford University.

Systematic position[3]
Order Rhabdocoela
Suborder Lecithophora
Section Dalyellioida
Family Umagillidae Wahl, 1910
Subfamily Umagillinae Wahl, 1910
Genus Syndisyrinx, gen. nov.
Genotype Syndisyrinx franciscanus, sp. nov.

Holotype. A whole mount in the United States National Museum, Washington D. C.

Repositories of type material. In each of the following repositories a whole mount, a transversely sectioned, and a sagittally sectioned preparation selected from the type material have been deposited: U. S. National Museum, Washington, D. C.; American Museum of Natural History, New York City; British Museum, London; California Academy of Science, San Francisco; Wilson Zoological Laboratory of the University of North Carolina; and Museum of Natural History, Stanford University. Additional preserved material may be obtained from the author or from any of these institutions.

Type locality. Mussel Point, Monterey Peninsula, California, Lat. 36°, 37′, 20″ N., Long. 121°, 54′, 15″ W.

Collectors. D. P. Costello, 1937 and H. E. Lehman, 1945.

Distinguishing characteristics. Umagillinae with a single intestine, paired and branched ovaries, cuticular penis, and a bursa seminalis connected by cuticular ducts to the seminal receptacle and bursal canal.

Materials and Methods

Fifty-four rhabdocoel parasites were obtained from two specimens of the sea urchin *Strongylocentrotus franciscanus* (A. Agassiz) by Dr. D. P. Costello in August 1937 at Pacific Grove, California. These specimens were fixed in Heath's, Boveri's, Lillie's and Worcester's solutions. Five of the individuals were sectioned serially at 10 μ and stained with Heidenhain's iron hematoxylin and orange G. One of these preparations was exceptionally fine and the majority of the accompanying figures were made from it. Unfortunately this preparation, which the author intended to designate as the holotype, was lost when a microscope was stolen. This material, including the slide preparations, was turned over to me by Dr. Costello. The morphological study was based on this material.

In the summer of 1945 during June, July, and August, the author collected several hundred additional specimens from the same locality. Over sixty urchins were examined and all were found to be infested; frequently three dozen or more parasites were obtained from the intestine of a single host. These worms were fixed in Heath's and Beauchamp's solutions. Seventy were sectioned serially at 10 μ and stained with Mayer's acid hemalum and triosin. Thirty whole mounts stained with paracarmine were also made. The type material was selected from these prep-

[3] Classification according to Bresslau (1933), with the exception of "Family Anoplodiidae Graff, 1913," which has been rejected in favor of "Family Umagillidae Wahl, 1910b," inasmuch as no reason is given by Graff for discarding the older name or for selecting *Anoplodium* as type genus. The subfamily Umagillinae has been retained as designated by Wahl, 1910b.

parations. At this time another parasite of *Str. franciscanus* was discovered which differed from *Syndisyrinx* in shape, manner of locomotion, and color. A description of this worm is being prepared and preliminary examination of sectioned material indicates a close relationship to *Syndesmis echinorum*. Upon the suggestion of Prof. A. R. Moore, who had occasionally observed parasitic worms in *Str. purpuratus* (Stimpson), forty-seven of these urchins were examined. In twenty-nine of them, worms that are very similar to, and may be identical with *Syndisyrinx franciscanus* were present in small numbers.

General Morphology

The living animals are bright red with a dark brown or yellow median longitudinal line which marks the extent of the intestine. The worms are flattened dorsoventrally and have a leaf-like appearance, being rounded at the anterior end and slightly pointed posterad. Individuals vary in size from 2 to 3 mm. long and 1.6 to 2.5 mm. wide. The body is thickest at approximately one-fourth of the distance from the anterior end and at this level measures about 0.5 mm. in the dorsoventral axis. Laterally and posteriorly the thickness of the body diminishes gradually to about 0.2 mm. at the periphery. A ciliated epithelium covers the entire surface; rhabdites and cuticle are lacking.

The mouth is situated on the ventral surface about one-fourth of the distance from the anterior end and a common genital pore opens ventrally at the posterior extremity of the body. The musculature and parenchyma are typical of other Umagillidae. No excretory system was observed. The strongly muscular pharynx is typically doliiform and possesses pharyngeal glands; it communicates by a short oesophagus with the gut. The intestine, possessing a number of small lateral diverticula, extends posterad under the dorsal epidermis along the mid-line and terminates one-quarter of the distance from the posterior end of the body. The gut contains no permanent lumen and food masses lie in temporary cavities surrounded by large digestive cells. The brain, composed of two cerebral ganglia connected by a wide commissure, lies anterior to the pharynx and gives off paired anterior, lateral, and posterior nerves.

Lobed testes lie lateral to the mid-line in the anterior half of the body. Accessory glands empty into the sperm duct that arises from each testis and passes anterad. These paired tubes unite mesially and enter a small spermiducal vesicle that is continued posterad as a muscular common sperm duct which lies dorsal to the uterus along the mid-line. This tube terminates in an elongated cuticular stylet, the penis, which is enlarged and funnel-like at the base. The penis stylet enclosed in the male antrum extends through the posterior third of the body to the common genital antrum and over most of its length does not exceed 3 μ in diameter.

Paired vitellaria are found immediately posterior to the testes; they are greatly ramified and fill most of the ventrolateral spaces in the middle third of the body. Posterior to the vitellaria a pair of ovaries is located, one on each side of the mid-line. Laterally each branches into five or more finger-like lobes. Three or four collecting ducts from the vitellaria empty with the ovaries and seminal receptacle into the anterior end of the ovovitelline duct. The seminal receptacle is oval and filled with sperm. Located posterodorsad to this organ is a vesicular, sperm-filled bursa seminalis connected to the seminal receptacle by a fine cuticular insemi-

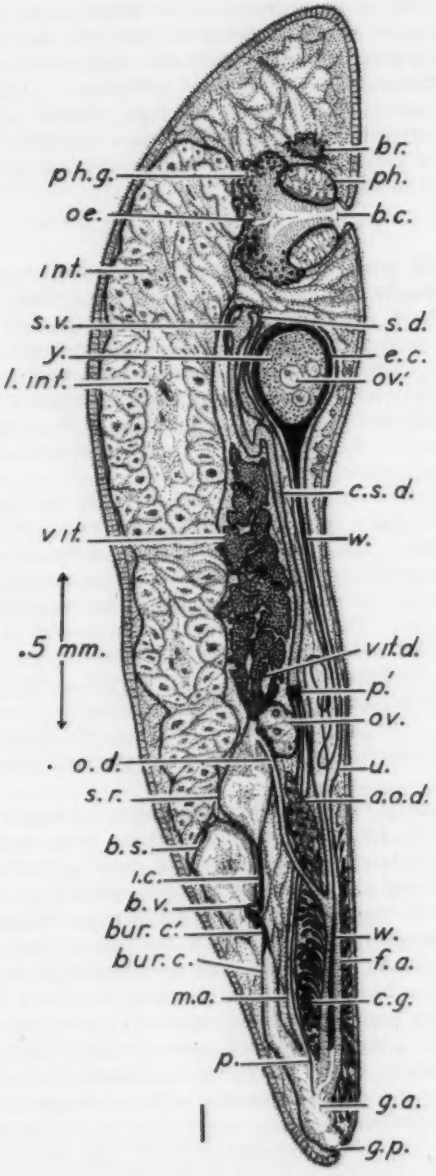


FIGURE 1. Semidiagrammatic median sagittal section.

nation canal. Arising in close association with this tubule is a similar duct, the cuticular proximal part of the bursal canal that passes posterad from the bursa seminalis approximately 60 μ before widening into the posterior muscular portion of the bursal canal (vagina). A cuticular sheath surrounds the openings of these two ducts into the bursa seminalis. The composite structure, consisting of this sheath and the canals passing through it, makes up the bursal valve.

An ovovitelline duct, into which accessory glands empty, arises ventrally at the anterior end of the seminal receptacle. It passes posterad and unites with the female antrum. The uterus, lying close to the ventral epidermis, extends anteriorly from the female antrum almost to the pharynx. At the anterior end of the uterus an egg capsule containing from one to five ova and numerous yolk cells is generally found. The capsule is continued posterad as a long coiled whip similar to those found in related forms. Most of the ventrolateral spaces of the posterior third of the body are filled by cement glands; they communicate by many small ducts with the female antrum. The common genital antrum is an elongated cavity at the posterior end of the body into which the female antrum enters ventrally, the male antrum and penis open mesially and the bursal canal is given off dorsally. At its posterior end is the common genital pore which opens ventrally to the exterior.

Histological Structure

Epidermis

A ciliated epithelium covers both dorsal and ventral surfaces of the body. No pigment or special gland cells were observed in this layer and a cuticle and rhabdites are lacking. The cytoplasm of the cells in the epidermal layer is granular and cell boundaries, though faintly stained, are distinct. The cells covering the dorsal surface are cuboidal and measure 10 μ from basement membrane to external surface. The cytoplasm of these cells stains moderately with hematoxylin. On the ventral surface the cells are flattened and are about 7 μ thick and from 12 to 35 μ wide; they have little affinity for hematoxylin. Cilia of the ventral epidermis are about 6.5 μ long and are almost twice the length of those found on the dorsal surface. Cells possessing the staining properties and short cilia characteristic of the dorsal layer extend for a short distance ventrally around the lateral edges. A zone 4 to 6 cells wide of intermediate nature accomplishes the transition between typical dorsal and ventral epithelium.

Musculature and parenchyma

The arrangement of the musculature is essentially the same as that described for other Umagillidae. Under the basement membrane of the surface epithelium is

---

Abbreviations for Figures 1 and 2.

a.o.d.—accessory glands of ovovitelline duct, a.s.d.—accessory glands of sperm duct, br.—brain, b.c.—buccal cavity, bur. c.—bursal canal, bur. c'.—cuticular end of bursal canal, b.s.—bursa seminalis, b.v.—bursal valve, c.g.—cement glands, c.s.d.—common sperm duct, e.c.—egg capsule, f.a.—female antrum, g.a.—common genital antrum, g.p.—genital pore, int.—intestine, i.c.—insemination canal, l. int.—lumen of intestine, m.a.—male antrum, oe.—oesophagus, ov.—ovary, ov'.—ovum, o.d.—ovovitelline duct, p.—penis, p'.—base of penis, ph.—pharynx, ph. g.—pharyngeal glands, s.d.—sperm duct, s.r.—seminal receptacle, s.v.—spermiducal vesicle, te.—testis, u.—uterus, vit.—vitellaria, vit. d.—vitelline ducts, w.—whip of egg capsule, y.—yolk cells.

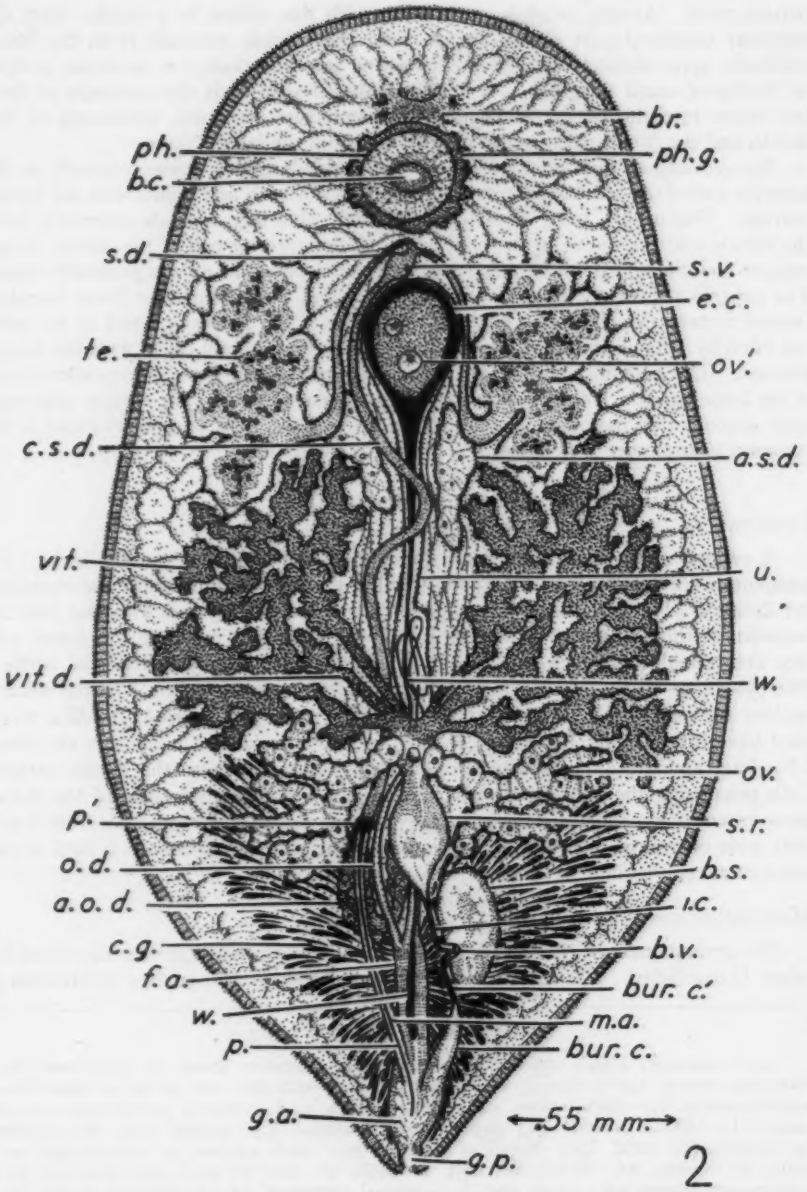


FIGURE 2. Semidiagrammatic median frontal section, intestine omitted.

found a thin layer of subepidermal muscles (Figs. 3–5, 7, 8). The superficial muscles are circular; these overlie a longitudinal sheet, and interposed at intervals between these layers are well-developed oblique fibers. In addition to these, bundles of fibers attached to the internal organs or the basement membrane of the epidermis pass dorsoventrally through the parenchyma (Figs. 1–3). The special muscles of the reproductive and digestive systems will be described in connection with the organs with which they are associated.

A parenchyma, composed of large, irregularly shaped cells with coarsely granular or vacuolated cytoplasm, fills most of the spaces between the internal organs and epidermis. A histologically distinct parenchymatous mass of cells enclosed in a fibrous capsule extends posterad along the mid-ventral line from the posterior level of the pharynx to the region in which the female antrum enters the common genital antrum. The flattened, nonvacuolated cells of this tissue possess finely granular cytoplasm and are arranged in concentric layers around the reproductive ducts, most of which pass through the mid-ventral parenchyma (Figs. 3–5, 7). Nowhere within the parenchyma were flame cells or collecting ducts of an excretory system observed.

Nervous system

The brain is similar in all respects to those described in other members of the family. It is located just anterior to the pharynx and consists of two ganglia connected by a wide commissure. Around the central fibrous mass of the brain are numerous ganglionic cells that stain quite evenly with hematoxylin. Poorly developed anterior, lateral and posterior pairs of nerves leave the brain and can be traced for short distances into the parenchyma. No theca separates the brain or nerves from the parenchyma and no special sensory organs were found.

Digestive system

The mouth lies on the ventral surface about one-fourth of the distance from the anterior end of the body. It opens into a very small buccal cavity lined by flattened ciliated cells that are continuous externally with the ventral epithelium (Figs. 1, 8). A sphincter underlying the epithelium regulates the size of the oral opening. Lying immediately dorsal to the mouth and opening into the buccal cavity is the doliiform pharynx which has the appearance of a dorsally compressed sphere. Its dorsoventral axis is about 0.1 mm. long and its greatest diameter is about 0.17 mm. Passing dorsoventrally through the pharynx is a funnel-shaped lumen that is narrowest at the oral or ventral end. The musculature of the pharynx is similar in most details of its organization to that found in *Syndesmis* as described by Russo (1895). A thin superficial layer of vertical fibers overlies the well-defined muscles encircling the lumen of the pharynx. In addition to the circular and vertical muscles, radial fibers pass from the lumen to the peripheral surface of the pharynx. Nonmuscular cells with heavily staining reticular cytoplasm fill the spaces between the radial fibers (Fig. 8). Surrounding the pharynx is a sharply defined basement membrane to which are attached numerous short, radially arranged, protractor muscles that extend to the basement membrane of the ventral epidermis. The more oblique of these fibers serve also as dilators of the pharynx. Poorly developed retractors are attached to the equator of the pharynx and pass to the dorsal surface. Pharyngeal glands are present encircling the dorsal end of the pharynx. The

peripheral contours of these glands are lobular and a thin basement membrane separates them from the parenchyma. The cells which make up these glands have indistinct cell boundaries and dense cytoplasm containing numerous granules that stain darkly with hematoxylin. Cytoplasmic continuations of the cells extend ventrally and line the lumen of the pharynx (Fig. 8). Leading dorsad from the pharynx is a short oesophagus which passes through the pharyngeal glands and opens into the anterior end of the intestine.

The intestine lies along the mid-line under the dorsal epidermis and extends posterad from the level of the brain to about one-fourth of the distance from the posterior end of the body (Fig. 1). The width of the gut varies from 0.1 to 0.2 mm. at the anterior end and diminishes gradually posteriorly. Short diverticula extend laterally on each side. The epithelium of the intestine is made up of large irregularly shaped cells containing moderately granular cytoplasm. The basal end of most cells reaches the fibromuscular investing sheath of the intestine that separates it from the parenchyma. The lumen of the intestine can only be observed when ingested material is present; this condition is similar to that found in some alloeocoels. In an animal that has been feeding, food masses often lie in cavities that have lost all direct communication with the oesophagus (Fig. 1). Food vacuoles of varying sizes are generally present in the cells surrounding the ingested material and digestive cells were occasionally observed that had apparently migrated into the food masses by amoeboid movement.

Male reproductive system

The paired testes lie lateral to the mid-line in the anterior half of the body. They are approximately 0.5 mm. long and from 0.3 to 0.5 mm. wide. Each is made up of four to six vesicular lobes, the lumina of which are in direct communication with one another (Fig. 2). Separating the testes from the parenchyma is a fibrous sheath that pentrates and partially subdivides the lobes. The chambers so formed are filled with developing germ cells and tangled masses of mature spermatozoa (Fig. 3). Mature sperm are present in all lobes but are more numerous midway between the anterior and posterior ends of the testes near the wide openings of the sperm ducts. These ducts run mesially from the testes and enter the mid-ventral parenchyma, whereupon they diminish to about 10 μ in diameter and generally continue their course anterad, dorsolateral to the uterus (Figs. 1–3). A thin epithelium surrounded by loose fibromuscular elements makes up the walls of the sperm ducts. Near the origin of these ducts from the testes, glandular cells that probably possess some accessory function are found in the mid-ventral parenchyma adjacent to the ventral walls of the tubes (Fig. 2).

At varying distances posterior to the pharynx the sperm ducts unite mesially and enter the anterior end of a common sperm duct which at this point is somewhat enlarged to form a small spermiducal vesicle (Figs. 1–3). The slightly coiled common sperm duct continues posterad from the vesicle through the mid-ventral parenchyma. It gradually diminishes in diameter from 45 μ to 12 μ. Its walls are composed of connective tissue cells surrounded by a sheath of circular, oblique, and longitudinal muscle fibers. The lumen of the tube is lined by a thin squamous epithelium that is separated from the theca by a thick basement membrane. Posteriorly, the common sperm duct unites with the enlarged base of the penis at

about one-third of the distance from the posterior end of the body (Figs. 1, 2, 4). The penis lies in a muscular sheath, the male antrum, which is a diverticulum of the genital antrum. Histologically this sheath is similar in most details of its structure to the common sperm duct; however, the lining epithelium of the male antrum is thicker, in some regions almost occluding the lumen, and a thick basement membrane is lacking (Fig. 5). The copulatory organ is a cuticular tubule that extends through the posterior third of the body and is about 3 μ in thickness over most of its length. The lumen of the stylet does not exceed 2 μ in diameter except at the anterior end of the penis which is enlarged to 12 μ at its union with the posterior end of the common sperm duct (Figs. 1–3). The rim of the funnel-like base of the penis is thickened to form a collar; longitudinal muscles in the walls of the male antrum and common sperm duct attach to this collar and function as protractors and retractors of the penis.

Female reproductive system

The paired ovaries lie in the posterior third of the body. Each is made up of from five to ten lobes that branch dichotomously from common trunks arising near the anterior end of the seminal receptacle. The lobes of the ovaries are directed posterolaterad and are separated from the parenchyma by a very poorly developed theca. The branches are made up of dovetailed chains or rouleaux of compressed ova that are proliferated from primordial cells at the distal ends of the lobes (Fig. 2). Mature ova are approximately 75 μ in diameter and vary in thickness from 20 to 60 μ. The cytoplasm of immature eggs is at first homogeneous, but as development continues many small peripherally distributed granules appear that are probably stored nutrient materials. During the period of growth the nuclei of the ova increase from 7 to 25 μ in diameter and the chromatin granules gradually lose their affinity for basic dyes. In mature ova only the spherical or oval nucleolus stains deeply with hematoxylin (Fig. 4).

A pair of greatly branched vitellaria lie anterior to the ovaries and fill most of the ventrolateral spaces in the middle third of the body (Figs. 1, 2). Many of the dorsoventral muscles of the parenchyma contribute fibers to the diffuse sheath that encloses these ducts. Primordial cells at the distal ends of the branches give rise to yolk cells. As the cells increase in size, the cytoplasm which at first is homogeneous, becomes filled with refractile granules that coalesce to form amber-colored droplets (Figs. 3, 4). From each side three or four collecting ducts packed with mature yolk cells pass posterad from the vitellaria and unite near the mid-line shortly before emptying into the anterior end of the ovovitelline duct (Fig. 2).

The seminal receptacle is somewhat oval and lies ventral to the intestine within the sheath that surrounds the gut. Its anterior extremity is about one-third of the distance from the posterior end of the body. The posterior part of this organ is thin walled and masses of mature spermatozoa are observable in its extensive lumen. Anteriorly the seminal receptacle opens with the paired ducts of the vitellaria and ovaries into the ovovitelline duct which arises ventrally in this region (Figs. 1, 2). The wall of the anterior third of the seminal receptacle is lined by large gland-like cells that restrict the lumen to a narrow channel 6 to 10 μ wide which connects the posterior vesicular portion to the ovovitelline duct (Fig. 4).

The bursa seminalis lies dorsal to the vesicular portion of the seminal receptacle. It is enclosed in the same sheath that surrounds the seminal receptacle and the pos-

terior end of the intestine (Figs. 1, 2). The large lumen of the bursa seminalis is lined by an epithelial layer very similar to that lining the posterior part of the seminal receptacle. In every specimen examined spermatozoa were found in the bursa; frequently they were aggregated into roughly spindle-shaped masses in which degenerating sperm were observable (Figs. 5, 6). Arising ventrally, or in some cases laterally, from the wall of the posterior half of the bursa seminalis is the insemination canal, a fine cuticular tubule about 4 μ in diameter connecting the lumina of the bursa seminalis and seminal receptacle. In close association with the insemination canal, a second cuticular tube of the same dimensions arises from the wall of the bursa seminalis and connects the bursa posteriorly to the bursal canal (Figs. 1, 2, 5, 6). Surrounding the ends of the ducts as they penetrate the lining epithelium of the bursa is a cuticular sheath, 7 μ in diameter and 10 μ long. The inner end of this sheath is involuted and fused to the ends of the two ducts (Fig. 6). To designate this composite cuticular structure made up of the insemination canal, the proximal end of the bursal canal and the sheath surrounding the ends of these ducts, the term, "bursal valve," is suggested.

The bursal canal (vagina) is a tubular structure about 0.1 mm. long and 20 μ in diameter that arises as an anterodorsal continuation of the common genital antrum. Its wall is composed of an inner epithelial layer surrounded by a strong fibromuscular sheath. At the posterior end of the canal the epithelium possesses cilia-like projections characteristic of the lining of the common genital antrum. Anteriorly the lumen of the canal is reduced and the thin basement membrane underlying the epithelium becomes continuous with the cuticular wall of the tubule leading into the bursa seminalis.

A flattened muscular ovovitelline duct (ductus communis) arises ventrally near the anterior end of the seminal receptacle and receives the ducts of the ovaries and vitellaria. It passes posterad through the mid-ventral parenchyma to about the level of the posterior end of the bursa seminalis and here enters the anterior end of the female antrum (Figs. 1, 2, 5). The ovovitelline duct is approximately 35 μ wide but is capable of considerable expansion to allow ova and yolk cells to pass into the uterus. Circular, oblique and longitudinal muscles are observable in contact with the thin basement membrane that underlies the lining epithelium; no fibrous sheath separates this duct from the cells of the mid-ventral parenchyma. Running parallel to the ovovitelline duct in the lateral parenchyma are paired accessory glands which enter the posterior part of the duct prior to its union with the female antrum (Figs. 1, 2, 5). Generally the cytoplasm of these gland cells stains evenly; however, in some cases the cells were observed to be filled with eosinophil granules.

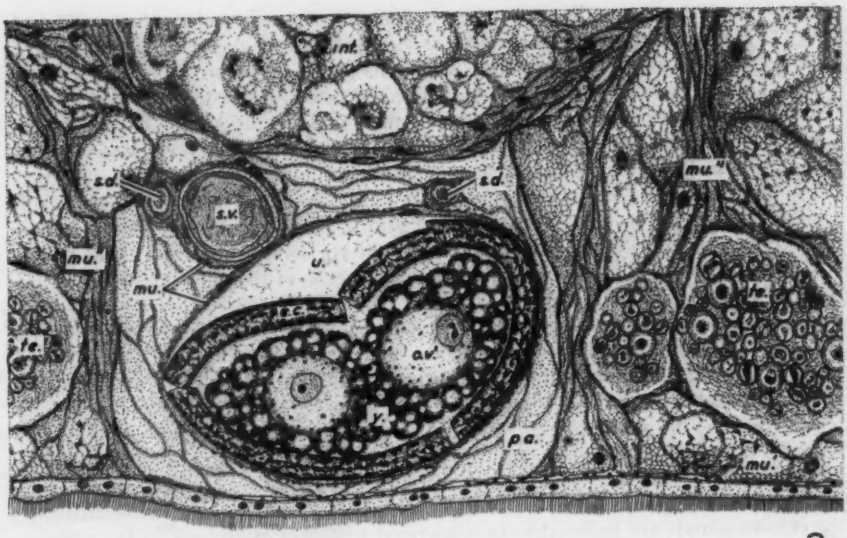
The uterus arises ventrally from the anterior end of the female antrum. It extends anterad almost to the pharynx through the mid-ventral parenchyma and

---

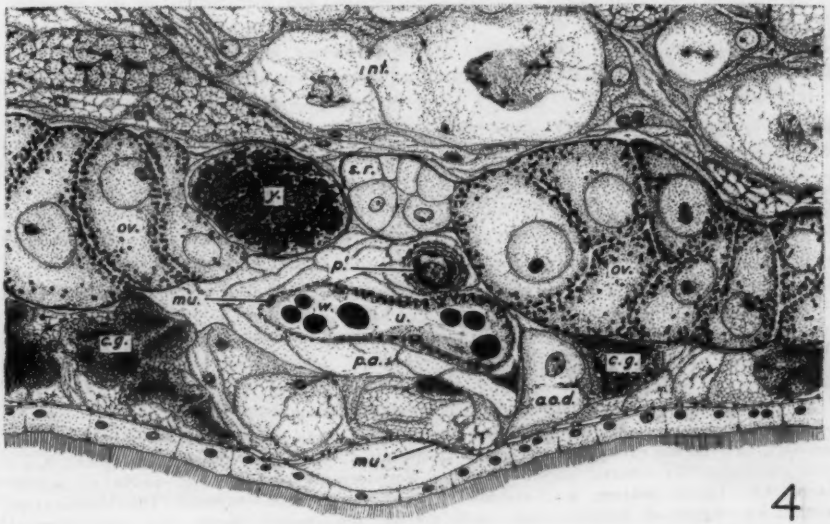
FIGURE 3. Transverse section through egg capsule and spermiducal vesicle (× 350).
FIGURE 4. Transverse section through entrance of ovary into seminal receptacle (× 500).

Abbreviations for Figures 3 and 4.

a.o.d.—accessory glands of ovovitelline duct, c.g.—cement glands, e.c.—egg capsule, int.—intestine, mu.—muscle sheath, mu'.—subepidermal muscles, mu''.—dorsoventral muscles of parenchyma, ov.—ovary, ov'.—ovum, p'.—base of penis, pa.—mid-ventral parenchyma, s.d.—sperm duct, s.r.—seminal receptacle, s.v.—spermiducal vesicle, te.—testis, u.—uterus, w.—whip of egg capsule, y.—yolk cells.



3



4

FIGURES 3–4.

through its entire course lies very close to the ventral surface of the body (Figs. 1, 2, 4). The anterior end of the uterus is enlarged and encloses an amber-colored, oval egg capsule containing numerous yolk cells and from one to five spherical eggs (Figs. 1, 2, 3). The egg capsule is cuticular and possesses a whip-like prolongation that extends posterad through the entire length of the uterus and female antrum. Over most of its length the whip is about 10 μ thick. In the middle portion of the uterus the whip is often coiled back upon itself a number of times so that its total length may greatly exceed that of the uterus (Figs. 1, 2). The uterine wall is very similar in structure to the ovovitelline duct and is able to enlarge greatly to accommodate the egg capsule and the folded part of the egg whip (Figs. 3, 4).

The female antrum extends from the posterior ends of the uterus and ovovitelline duct to the common genital antrum (Figs. 1, 2). The walls are lined by columnar epithelial cells surrounded by a thin basement membrane and a muscular layer that is continuous with the fibers enclosing the uterus and ovovitelline duct. The lumen is about 12 μ in diameter and the posterior end of the egg whip, when present, almost completely fills this space (Figs. 5, 7). The ventrolateral spaces of the posterior third of the body contain numerous unicellular cement glands. The cytoplasm of these cells is generally uniformly filled with small granules that have a strong affinity for hematoxylin. Throughout the entire length of the female antrum many ducts from these glands enter the lateral walls (Figs. 1, 2, 5, 7). The secretions of the cement glands are believed to be associated with the attachment of the egg capsules to the substrate when expelled. Living animals compressed under a cover glass were occasionally observed at low magnification to undergo a series of rapid contractions which resulted in the extrusion of the egg capsule and whip. However, nothing is known about the normal deposition and attachment of the capsules, nor are other details of the life cycle understood.

The common genital antrum lies at the posterior end of the body. It is an elongated tube lined by flattened cells that appear to have cilia about 20 μ long which extend into the lumen (Figs. 1, 2, 7). A diffuse fibrous sheath separates this organ from the parenchyma. The common genital antrum receives the terminal ducts of both male and female reproductive systems: the bursal canal arises from it as a dorsal diverticulum; the male antrum enclosing the penis stylet is given off as a mesial evagination; and the female antrum enters it ventrally. The common genital pore opens on the ventral surface at the posterior end of the body. At this point

---

FIGURE 5. Transverse section through bursa seminalis and bursal valve (× 350).

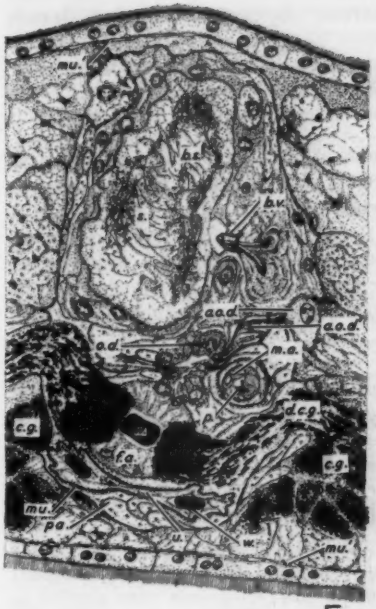
FIGURE 6. Bursal valve (× 1,050).

FIGURE 7. Transverse section through the entrance of female antrum and male antrum into the common genital antrum (× 350).

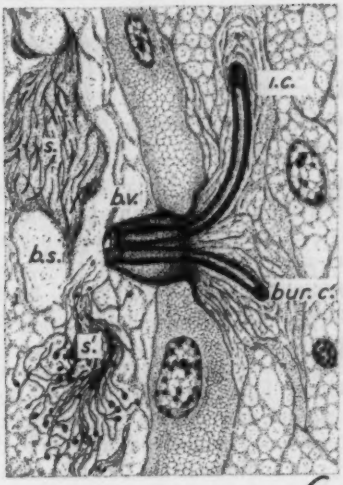
FIGURE 8. Transverse section through pharynx (× 200).

Abbreviations for Figures 5 through 8.

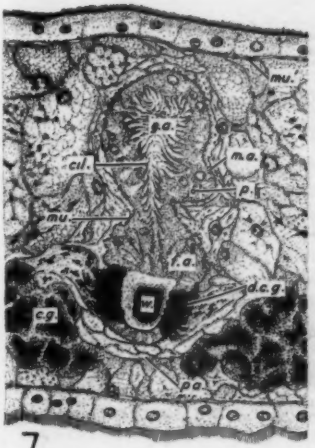
a.o.d.—accessory glands of ovovitelline duct, a.o.d′.—ducts of accessory glands of ovovitelline duct, b.c.—buccal cavity, bur. c.—bursal canal, bur. c′.—cuticular end of bursal canal, b.s.—bursa seminalis, b.v.—bursal valve, cil.—cilia, c.g.—cement glands, d.c.g.—ducts of cement glands, f.a.—female antrum, g.a.—common genital antrum, int.—intestine, i.c.—insemination canal, l. int.—lumen of intestine, m.a.—male antrum, mu.—muscle sheath, mu′.—subepidermal muscles, mu″.—pharyngeal protractor muscles, n.—nerves, oe.—oesophagus, o.d.—ovovitelline duct, pa.—mid-ventral parenchyma, p.—penis, ph.—pharynx, ph. g.—pharyngeal glands, s.—spermatozoa, s′.—degenerating spermatozoa, u.—uterus, w.—whip of egg capsule.



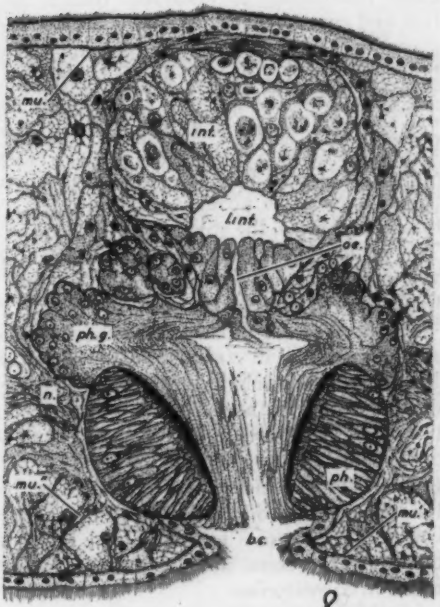
5



6



7



8

FIGURES 5–8.

the ciliated ventral epithelium is invaginated and forms a short bulb-like canal which meets an outpocketing of the common genital antrum. Sphincters encircle both ends of this canal and regulate the size of the pore.

Discussion

Comparison of genera

Although the parasite described here is similar in many respects to all genera in the family Umagillidae, there are certain structural characteristics that do not correspond to those of any previously reported genus of this family. Therefore, it is considered necessary to establish a new genus to be designated by the name *Syndisyrinx*. This name is intended to describe the complex bursal valve which is not present in any other genus of the family. The specific name, *Syn. franciscanus,* is given to designate the host, *Strongylocentrotus franciscanus,* in which it was first found.

For the sake of uniformity in the following comparison of genera of the family Umagillidae, the morphological nomenclature used by the various authors in their original descriptions of genera and species has been altered to conform with the terminology employed in the preceding analysis of *Syndisyrinx*.

In addition to the fact that both *Syndisyrinx* and *Syndesmis* are found in the intestine of echinoids, the morphological characteristics of *Syndisyrinx* indicate a closer relationship to *Syndesmis* than to the other genera of the family. The location and appearance of important organs, *viz.,* muscular pharynx, lobed testes, small spermiducal vesicle, muscular common sperm duct, ramified vitellaria, dichotomously branched ovaries, elongated uterus and egg capsule with whip, are very similar in *Syndesmis* and *Syndisyrinx* and strongly suggest a close relationship between these two genera. *Syndisyrinx* differs from *Syndesmis* chiefly in the structure and relationships of the bursa seminalis and seminal receptacle. In *Syndesmis* a single vesicle is present for the reception of sperm and cuticular structures such as the parts which make up the bursal valve of *Syndisyrinx* are lacking. In addition to these differences, the stucture of the penis is markedly dissimilar in these two forms. The penis of *Syndisyrinx* is a cuticular hollow stylet attached only at the base, whereas the copulatory organ of *Syndesmis* is a muscular eversible tube with a cuticular lining (Russo, 1895; Fig. 16).

Structures corresponding to the cuticular canals in the bursal valve of *Syndisyrinx* are found in *Anoplodiera voluta, Wahlia macrostylifera,* and *Desmote vorax*. In *A. voluta* the relationships of the two cuticular canals to the bursa seminalis, as described by Westblad (1930), are very similar to the arrangement of these structures in *Syndisyrinx*. However, the cuticular sheath that surrounds the entrance of these ducts into the bursa is lacking in *A. voluta*. There do not appear to be grounds for concluding that *Syndisyrinx* and *Anoplodiera* are closely related since the appearance and location of the testes and vitellaria, the presence of a single ovary, and the absence of a female antrum connecting the ovovitelline duct and uterus to the common genital antrum in *A. voluta* differ strikingly from the arrangement found in *Syndisyrinx*.

In *W. macrostylifera,* described by Westblad (1930), and *D. vorax,* according to Beklemischev (1916), the proximal end of the bursal canal is cuticular but an insemination canal is lacking. In other respects *W. macrostylifera* differs from

Syndisyrinx chiefly in regard to the morphology of the male reproductive system. The penis stylet is greatly elongated, and paired sperm ducts arising from compact testes unite and communicate by means of a single duct with the large spermiducal vesicle situated anterior to the pharynx. Many points of difference are likewise found by comparing the morphology of *Syndisyrinx* and *Desmote*. The most evident of these are the bipartite gut and the presence of two genital pores, the anterior pore by which the uterus opens to the exterior and the posterior pore which serves for copulation in *D. vorax*.

The other genera of the family lack cuticular parts in the copulatory complex comparable to those in the bursal valve of *Syndisyrinx* and to a greater or less degree exhibit dissimilarities in the location, distribution, number, arrangement and relationships of organs in the body. In these genera the most conspicuous differences with respect to *Syndisyrinx* are: the single ovary and absence of a cuticular copulatory stylet in the genus *Anoplodium;* the unbranched ovaries and double-walled cuticular penis stylet in the genus *Umagilla;* the absence of a cuticular penis and the general arrangement of testes and vitellaria in the genus *Xenometra;* and the single testis in the genus *Collastoma.* A manuscript is in preparation which will deal at greater length with the structural relationships of these forms.

Bursal valve

There is a superficial similarity between the bursal valve of *Syndisyrinx* and the cuticular nozzle-like mouthpieces of acoels. In the acoel, *Amphichoerus,* described by Graff (1891), and many allied forms, one end of the mouthpiece is generally connected to a vesicular sac or bursa filled with sperm; the other end is directed toward the ovary. L. H. Hyman (1937) points out that the function of these mouthpieces is apparently to direct sperm toward the ova to help insure fertilization. This function can hardly be ascribed to the insemination canals of *Anoplodiera* and *Syndisyrinx* which conduct sperm from the bursa seminalis to the seminal receptacle and not directly to the ova; nor does it seem probable that the insemination canals of Umagillidae are homologous to these mouthpieces. Noncuticular ducts connect the bursa seminalis to the seminal receptacle and bursal canal in most genera of Umagillidae, which suggests that cuticular structures are probably of relatively recent rather than primitive origin. In an analysis of the existing genera, Wahl (1910b) presents evidence which leads him to conclude that *Umagilla* is the most primitive and least modified genus of the family. If one accepts this view, it lends support to the opinion expressed above, inasmuch as *Umagilla* lacks any cuticular structures that might be considered homologous to the bursal valve. It is possible that the absence of cuticular parts in some of the species is due to a greater degree of simplification associated with a parasitic existence. However, there is no direct evidence for this supposition, since in the most closely related free-living families, Graffillidae and Dalyelliidae, cuticular structures such as these are not found. This suggests that these tubules have arisen independently, and until additional information is available, the insemination canals and bursal canals of Umagillidae should not be considered as mouthpieces in a true sense.

Although copulation has not been observed in *Syndisyrinx,* it is believed that the sperm of one animal are injected by means of the protrusible penis into the bursal canal of another. Before fertilization can take place, sperm must migrate from the

bursal canal through its narrow proximal end into the bursa seminalis, there remaining until able to find their way through the insemination canal into the seminal receptacle. Evidently many sperm are unable to accomplish this migration and degenerate in the lumen of the bursa seminalis. Sperm that do reach the seminal receptacle must then pass through the constricted anterior part of this organ to fertilize the mature ova that enter the ovovitelline duct at the anterior end of the seminal receptacle.

It is difficult to explain any selective advantage for the presence of the fine canals that make up the bursal valve of *Syndisyrinx*. It was thought at first to be a mechanism for the prevention of polyspermy. However, this explanation is negated by the presence of large masses of spermatozoa in the seminal receptacle. The simplest explanation for the presence of these ducts is that they act as valves which regulate the number of spermatozoa entering the bursa seminalis and seminal receptacle. If this interpretation is correct, it is probable that the function of the bursal valve is to insure a necessary aging of the sperm in the bursa before fertilization. The cuticular walls are necessary to prevent the collapse of these narrow tubes. It is evident that the bursal valve restricts the free passage of sperm from the bursa seminalis and therefore as the result of a single copulation, a continuous supply of sperm may be maintained over a long period of time.

Summary

After completing a histological study of an endoparasitic rhabdocoel from the Pacific Coast sea urchin, *Strongylocentrotus franciscanus*, the following conclusions have been reached:

1. This parasite belongs to the rhabdocoel family Umagillidae but differs in certain characteristics from the eight known genera of the family.
2. The distinguishing characteristics are a single intestine, paired and lobed ovaries and testes, a tubular single-walled cuticular penis stylet, and cuticular ducts connecting the bursa seminalis to the bursal canal and seminal receptacle.
3. A characteristic structure typical of this parasite and not present in other genera of the family is the bursal valve composed of two cuticular tubes, the insemination canal and proximal end of the bursal canal, which enter the bursa seminalis through a cuticular cup-like sheath.
4. The parasite here described is given the name *Syndisyrinx franciscanus, gen. et sp. nov.*

LITERATURE CITED

Beklemischev, W., 1916. Sur les Turbellariés parasites de la côte Mourmanne, II. Rhabdocoela. *Trav. Soc. Imp. Nat. Petrograd, Zool. et Physiol.*, Sect. 4, **45**: 1–59 (Résumé, 60–79).

Bock, S., 1926. Anoplodium stichopi, ein neuer Parasit von der Westküste Skandinaviens. *Zool. Bidrag, Uppsala*, **10**: 1–30.

Braun, M., 1889. Über parasitische Strudelwürmer in Rostok. *Centralbl. Bakt. Parasit.*, Abt. 1, **5**: 41–44.

Bresslau, E., 1933. Turbellaria. *Kükenthal und Krumbach, Handbuch der Zoologie*, **2**: 264–269.

Dörler, A., 1900. Neue und wenig bekannte rhabdocöle Turbellarien. *Zeitschr. wiss. Zool.*, **68**: 1–42.

FRANCOIS, P. H., 1886. Sur le Syndesmis, nouveau type de Turbellariés décrit par, W. A. Silliman. *C. R. Acad. Sci. Paris,* **103**: 752–754.

GRAFF, L. v., 1891. *Die Organisation der Turbellaria acoela,* Leipzig (Engelmann), p. 73.

GRAFF, L. v., 1913. Turbellaria, II. Rhabdocoelida. *Das Tierreich,* Berlin (F. E. Schulze), **35**: 152–163.

HYMAN, L. H., 1937. Reproductive system and ćopulation in Amphiscolops langerhansi (Turbellaria acoela). *Biol. Bull.,* **72**: 319–326.

OZAKI, Y., 1932. On a new genus of parasitic Turbellaria, Xenometra, and a new species of Anoplodium. *Jour. Sci. Hiroshima Univ.* (*Series B, Zool.*), **1**: 81–89.

POWERS, P. B. A., 1936. Studies on the ciliates of sea urchins. A general survey of the infestations occurring in Tortugas echinoids. *Pap. Tortugas Lab. Carnegie Inst. Washington,* **29**: 319–320.

RUSSO, A., 1895. Sulla morfologia del Syndesmis echinorum Francois. *Ricerche Lab. Anat.,* Roma, Fasc. 1, **5**: 43–68.

SCHNEIDER, A., 1858. Über einige Parasiten der Holothuria tubulosa. I. Anoplodium parasita. *Müller's Arch. f. Anat. Phys. und wiss. Med.,* Berlin: 324–329.

WAHL, B., 1909. Untersuchungen über den Bau der parasitischen Turbellarien aus der Familie der Dalyelliiden (Vorticiden). II. Teil, Die Genera Umagilla und Syndesmis. *Wien, Sitz.-Ber. kais. Akad. wiss. Math.-nat.,* Abt. 1, **118**: 943–965.

WAHL, B., 1910a. Untersuchungen über den Bau der parasitischen Turbellarien aus der Familie der Dalyelliiden (Vorticiden). III. Teil, Das Genus Collastoma. *Wien, Sitz.-Ber. kais. Akad. wiss. Math.-nat.,* Abt. 1, **119**: 363–391.

WAHL, B., 1910b. Beiträge zur Kenntnis der Dalyelliiden und Umagilliden. *Festschr. f. R. Hertwig,* Jena (G. Fischer), **2**: 41–60.

WESTBLAD, E., 1926. Das Protonephridium der parasitischen Turbellarien. *Zool. Anz.,* **67**: 323–333.

WESTBLAD, E., 1930. Anoplodiera voluta und Wahlia macrostylifera, zwei neue parasitische Turbellarien aus Stichopus tremulus. *Zeitschr. f. Morph. u. Ökol. Tiere,* **19**: 397–426.

A QUANTITATIVE STUDY OF THE RELATIONSHIP BETWEEN THE ACTIVITY AND OXYGEN CONSUMPTION OF THE GOLDFISH, AND ITS APPLICATION TO THE MEASUREMENT OF RESPIRATORY METABOLISM IN FISHES

W. A. SPOOR

Department of Zoology, University of Cincinnati

Introduction

The fact that fish consume more oxygen when active than when quiescent has been observed by many investigators (Krogh, 1916; Bowen, 1932; Clausen, 1933, 1936; Wells, 1935; Schlaifer, 1938; Smith and Matthews, 1942), but apparently no attempt has been made to determine the exact relationship between oxygen consumption and activity in fishes. It is the purpose of this paper to present data which are believed to provide an objective and quantitative basis for the relationship between activity and oxygen consumption in the goldfish, and to describe a method for making the necessary measurements. The method is based on the use of a recording activity detector (Spoor, 1941) combined with a continuous flow system for measuring oxygen consumption.

The lack of definite information on the activity of fish under experimental conditions has been one of the chief sources of difficulty in work on the respiratory metabolism of fishes, and attention has been called to the need for an experimental method which would make it possible to distinguish between "standard metabolism" and the increased metabolism due to muscular movements (Wells, 1935). In view of the fact that the oxygen consumption is affected by changes in the basal metabolic rate as well as by changes in activity, the importance of such a method is apparent. The method employed in the present work seems to meet this need, inasmuch as the state of activity is recorded continuously and periods of inactivity can be selected for measuring basal oxygen consumption.

Szymanski (1914) and Spencer (1939), using other types of activity detectors, have reported that goldfish show considerable individual variation in activity and that the activity pattern is affected by light. Spencer (1939) also found activity to be influenced by food. Knowledge of the behavior of the fish under the experimental conditions is of importance in the collection of data on oxygen consumption in the method to be described, as well as in the interpretation of these data. For this reason further observations on the patterns and rates of activity and on the effects of food, light and disturbances are included in the present paper.

The Activity of the Goldfish Under Experimental Conditions

Method

Several dozen goldfish (*Carassius auratus*) ranging between 24 and 96 grams in weight were selected at random from a stock obtained from a local goldfish farm

and studied individually in experimental chambers, each of which was equipped with a recording activity detector. The experimental chambers were set up in a ground floor aquarium room which was seldom entered except for the purposes of this study, so that the fish could be left for long periods with relatively little disturbance. The recording apparatus was kept in another room. Records of the activity of each fish were started shortly after its introduction into a chamber and continued for periods ranging from a few days to many months in length, during which the patterns and rates of activity and the effects of food, light and disturbances upon them were studied. With a few exceptions, oxygen consumption was not measured in this series of observations.

The experimental chamber (Fig. 1) consisted of a one-gallon brown glazed

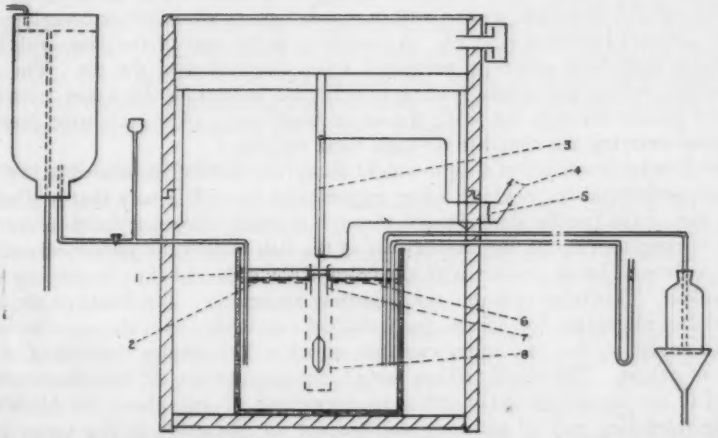


FIGURE 1. Diagram of apparatus for measuring oxygen consumption and activity. (1) paraffin oil (this was omitted when activity alone was being measured), (2) glass plates, (3) No. 44 copper wire, (4) to sensitive relay, (5) resistor, (6) wire screen, (7) glass tube, (8) wire frame protecting paddle. Explanation in text.

crock fitted with a galvanized iron wire screen of ½ inch mesh to prevent the fish from reaching the surface of the water. A glass tube about 3 cm. in diameter was fitted into an opening in the center of the screen so that it extended 3.5 cm. above and 3 cm. below the screen; its purpose will be considered in a later section. The surface of the water stood about 3 mm. above the screen, the total volume to this level being 2,600 cc. Water entered the chamber from a constant level reservoir through 8 mm. glass tubing and left by way of a siphon of 8 mm. glass tubing which dipped into a constant level drain, the rate of flow (between 70 and 100 cc. a minute) being regulated by means of a glass stopcock in the inlet. The intake of the siphon was placed about 5 cm. above the bottom of the chamber, so that feces and other debris that fell to the bottom did not enter the siphon until they had been broken into small pieces in the course of their passage upward to the intake. The chamber was practically self cleaning under these conditions, the flow of the water and the movements of the fish being sufficient to move debris into the siphon. The fish

could therefore be maintained in the chamber for months without cleaning. A thistle tube entering the inlet provided for the introduction of food, being closed off at all other times. The water supply consisted of tap water passed through an activated charcoal filter, brought to the desired temperature and aerated until it approached equilibrium with the atmosphere. Most of the observations were made at temperatures between 20 and 24° C. The fish seldom extracted more than one-third of the oxygen from the water at the rates of flow employed, and they usually took less than this. In view of the findings of Crozier and Stier (1925), Toryu (1927), and Schlaifer (1938), it seems unlikely that behavior was influenced by the oxygen tension of the water.

The chamber was enclosed in a wooden case to minimize disturbances and to make it possible to control the light. The top of the case was fitted with a pane of glass for natural illumination, and with a wooden cover when either complete darkness or constant light was desired. A ventilator in the side of the case, with baffles to prevent light from entering, permitted some circulation of the air. The water inlet, outlet siphon, and a tube leading to a U tube indicating the water level in the chamber passed through the wall; a coat of black paint over each tube prevented light from entering the chamber through these openings.

The detector consisted of a light-weight aluminum paddle suspended in the water in the experimental chamber by a fine copper wire in such a way that a silver rod at the top of the paddle shaft passed through a small hole in a fixed silver plate. Water currents set up by the movements of the fish moved the paddle, causing the rod to make and break contact with the sides of the hole and thus to activate a sensitive relay. This relay operated the recording apparatus. The blade of the paddle consisted of aluminum foil (5 cm. long and 2.5 cm. wide) with the corners bent in at right angles so that the water currents struck a flat surface regardless of their points of origin. The shaft (10 cm. long) consisted of no. 22 aluminum wire cemented to the blade and imbedded at its upper end (7 cm. above the blade) in a bakelite insulating rod (2 cm. long and 0.2 cm. in diameter) in the upper end of which the silver rod (1 cm. long and about 0.04 cm. in diameter) was imbedded. This silver rod was soldered to a 14.5 cm. length of no. 44 enameled copper wire held in an insulated binding post attached to a wooden supporting shaft. A wooden bracket rising from the case supported this shaft in a vertical position so that the paddle hung in the water through the glass tube in the center of the screen. A cylindrical frame of galvanized iron wire protected the paddle from contact with the fish. A small lead weight (about 0.1 gm.) clamped to the paddle shaft below the bakelite helped to bring the paddle back to the resting position after displacement by the water currents. The silver plate (about 0.5 cm. square) was attached to the supporting shaft and held in a horizontal position about 6 cm. above the screen. The hole in the plate was between 0.08 and 0.1 cm. in diameter. The current to operate the sensitive relay was supplied by a 6 volt storage battery; the coil of the relay had a resistance of 1,000 ohms. A 5,000 ohm resistor across the detector contacts prevented sparking and welding without causing an observable reduction in the sensitivity of the detector. The plate was kept warm by means of a small insulated heating coil in order to prevent water from condensing upon it from the humid atmosphere above the chamber.

The sensitivity of the detector could be controlled somewhat by adjusting the position of the silver rod with respect to the sides of the hole in the plate. The nor-

mal movements of the operculum and the position-maintaining fin movements of a quiescent 25- to 30-gram goldfish were usually sufficient to move the paddle slightly, and when the rod was close to the plate these movements were recorded. For the observations to be described, however, the rod was centered in the hole so that the ordinary respiratory movements did not move the paddle enough to make contact, these movements being considered as among the basal functions of the fish. Vigorous respiratory movements and any movement that resulted in a change in the position of the fish moved the paddle enough to make and break contact, slow swimming movements causing few, and vigorous activity causing many impulses to be recorded. At the flow rates used in these experiments the flow of water through the chamber did not move the paddle.

The sensitive relay activated a counter which in turn caused signal magnets to record every tenth and hundredth impulse on a long paper kymograph moving about 30 mm. an hour. The frequency of the impulses was such (ranging up to 6,000 an hour) that they usually could not be counted when recorded individually. The counter was capable of following and recording at least 10 impulses a second. Time was recorded in hours beneath the activity record.

Patterns and rates of activity

In agreement with the results of Szymanski (1914) and Spencer (1939), the goldfish used in this study proved to be quite variable in their patterns and rates of activity, even when they were maintained under almost identical conditions of light, feeding, temperature, water supply and disturbance. Three general types of behavior appeared when the fish were kept under natural conditions of light: (1) arhythmic activity, in which no relation to day or night could be detected; (2) rhythmic activity, in which the fish were active by day and quiescent at night; (3) rhythmic activity, in which they were quiescent by day and active at night.

Fish showing the first, arhythmic, type of behavior were extremely variable. A few were vigorously active day and night for periods as long as ten days, others were moderately active throughout the 24-hour period for weeks at a time, and still others remained practically inert for similar periods. Some of these arhythmic fish, particularly those in the last group, showed irregular bursts of activity now and then, with no apparent relation to the time of day, feeding, or disturbance.

An example of the second type of behavior, diurnal activity and nocturnal quiescence, is shown in Figure 2, which is based on the number of impulses recorded by a 35-gram male goldfish during each hour between 1 P.M. January 11 and 1 P.M. January 13, 1946. The fish was fed at 11:05 A.M. on January 12, otherwise the room was not entered between 2:45 P.M. January 11 and 7:15 P.M. January 13. Aside from the feeding, the effects of which are discussed below, light was the only known variable, the temperature, rate of flow and aeration of the water being the same at the end as at the beginning of the period. Most of the fish showing rhythmic changes in activity followed patterns of this type, although the active phase varied considerably, sometimes being interrupted by several hours of quiescence during the day, sometimes beginning later in the day, and occasionally continuing well into the night. The most constant period of quiescence occurred between midnight and 4 A.M., which is in agreement with Spencer's (1939) observations.

The third type of behavior, diurnal quiescence and nocturnal activity, was found less frequently than the second, although it was not uncommon. An example is

shown in Figure 3, which is based on records of the activity of a 32-gram male goldfish between 1 P.M. July 3 and 1 P.M. July 5, 1945. The room was not entered between 4 P.M. July 3 and 8 A.M. July 5, and aside from the daily changes in light the environmental conditions apparently remained constant throughout the period.

The patterns of rhythmic fish did not seem to be fixed, however, even when the environmental conditions remained unchanged. After several weeks of rhythmic behavior the fish frequently became arhythmic for several weeks or months, occasionally becoming rhythmic again in the course of extended periods of observation. This suggests that those fish which did not show daily activity rhythms under the

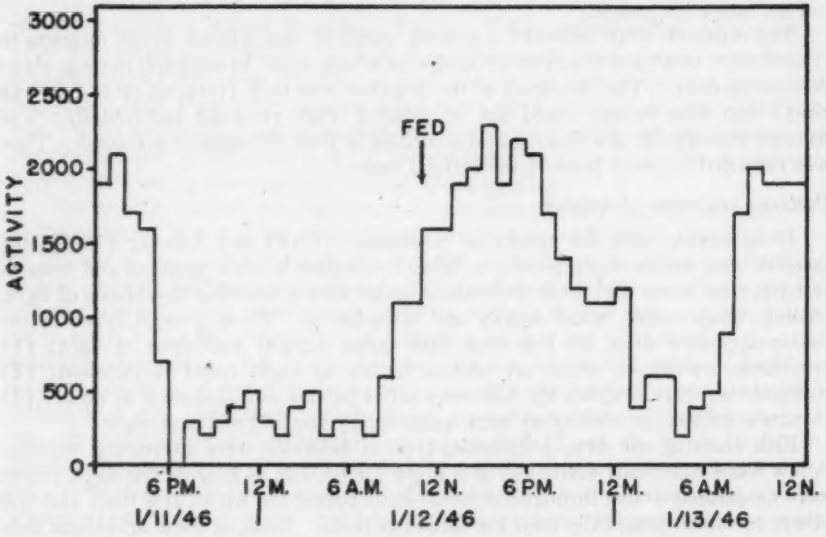


FIGURE 2. Activity pattern of 35-gram male goldfish between 1 P.M. January 11 and 1 P.M. January 13, 1946. Activity is expressed as number of impulses recorded each hour. Temperature 21.5° C. Fed at 11:05 A.M. January 12.

experimental conditions may have done so eventually had they been studied for longer periods, and that by chance the observations were made during arhythmic periods.

Activity and food

The fish were fed rolled oats, commercial fish foods, shredded shrimp, ground liver or chopped earthworms about three times a week, usually 0.5 to 1 gram at each feeding. The effects of daily feeding, larger amounts of food and starvation were also studied. Under the conditions of the experiments the type of food given had no consistent effects upon activity, but the quantity of food had pronounced effects, particularly on the total amount of activity. A well fed fish was usually sufficiently active that the number of impulses recorded in the course of a 24-hour period averaged between 500 and 1,500 an hour, and averages in excess of 2,500 impulses an

hour were not uncommon. Starvation caused this rate to decrease markedly, sometimes to fewer than 100 impulses an hour, although as a rule the lowest rates did not appear until the fish had been starved for a week or so. No fish was observed to become completely inactive for periods of more than an hour or two, however, even when starved for two weeks. The effects of feeding after a period of starvation were striking, activity increasing to normal "well fed" rates within a few minutes. Doubtless the swimming movements associated with feeding accounted for some of the activity recorded following the introduction of food, but it seems that the nutritional state also affected the amount of activity. Food given in amounts of one

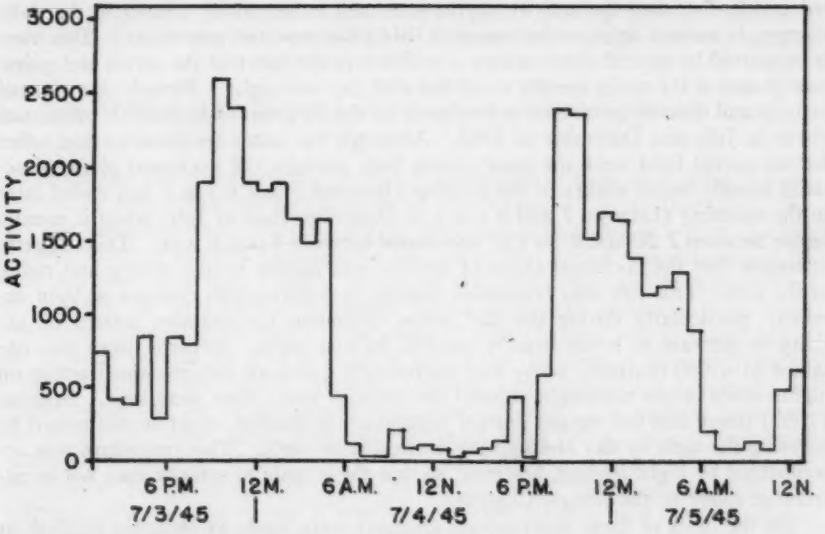


FIGURE 3. Activity pattern of 32-gram male goldfish between 1 P.M. July 3 and 1 P.M. July 5, 1945. Units as in Figure 2. Temperature 23.5° C.

gram or less was usually consumed within three to six hours, but the fish remained active (in accordance with their activity patterns) for from several days to a week after they had been fed. Similarly, Spencer (1939) found the goldfish to maintain a high rate of activity for several hours after feeding, although in his experiments the food was usually consumed within 15 minutes or less.

The effects of feeding upon activity rhythms were not studied in detail, but the available data bearing on this question indicate that although the rhythms appearing under natural conditions of light were frequently modified by the quantity of food and the time of feeding, they were not causally related to food. Feeding modified the activity patterns of some fish for part or all of the subsequent 24-hour period, usually by prolonging the active phase. A response of this type may be seen in Figure 2. The fish was fed 0.5 gm. of rolled oats at 11:05 A.M. on January 12 (the previous feeding being on January 9); it will be noted that the activity level remained relatively high for a much longer period on the night of January 12 than on

the preceding night. On the other hand some fish showed no change in activity in response to feeding, provided of course that they had not been starved. Variations in the quantity of food and in the time and frequency of feeding did not seem to have permanent effects on the activity rhythms, and feeding at the same time each day did not cause arhythmic fish to become rhythmic.

Activity and light

The goldfish did not seem to be much affected by changes in light intensity while they were not following daily activity rhythms, but they were usually quite responsive to light during their periods of rhythmic behavior. In fact, when the fish were well fed and undisturbed the activity rhythms seemed to be closely related to the daily changes in natural light, as Szymanski (1914) has reported previously. This view is supported by several observations in addition to the fact that the active and quiescent phases of the cycles usually coincided with day and night. Periods of nocturnal activity and diurnal quiescence were shown by the 32-gram male goldfish mentioned above in July and December of 1945. Although the water temperature and other factors except light were the same during both periods, the nocturnal phase of activity usually began earlier in the evening (between 5 and 6 P.M.) and ended later in the morning (between 7 and 8 A.M.) in December than in July, when it usually began between 7:30 and 8:30 P.M. and ended between 5 and 6 A.M. This suggests of course that the nocturnal phase of activity was limited by the setting and rising of the sun. This fish also responded readily to experimental changes in light intensity, particularly during the day, when darkening the chamber caused its activity to increase to levels usually reached only at night. Records were also obtained in which diurnally active and nocturnally quiescent fish remained active on nights when bright moonlight entered the room in which they were kept. Spencer (1939) found that the regular diurnal rhythm of the goldfish could be obliterated by covering the tank by day and lighting artificially at night. This procedure was accompanied by night feeding, however, so that the change in activity may not be attributed solely to the reversed lighting.

On the basis of these observations attempts were made to maintain goldfish at definite rates of activity by exposing them to continuous dim light and to continuous darkness for periods lasting as long as three weeks, but without success. The fish did not maintain constant rates of activity under either condition, but continued to alternate periods of increased activity with periods of relative quiescence. In order to maintain a low rate of activity it was necessary to starve the fish for about a week, the relationship between nutritional state and amount of activity being similar to that described in the preceding section.

Activity and disturbance

The goldfish proved to be extremely sensitive to disturbances. Noise, slight changes in the water level, sudden lights, the mere presence of the observer in the room, or such minor disturbances as the quiet opening and closing of the door to the room usually caused a change in the rate of activity. Fish that had been active before the disturbance almost invariably became less active, sometimes practically motionless, while quiescent fish frequently, although less consistently, became active when disturbed. Whichever the response, the original state of activity was

usually resumed within a few minutes after the disturbance had ceased. The degree of response seemed to be related to the amount of disturbance, for when the observer moved slowly and quietly the change in activity was usually less pronounced, and recovery more rapid, than after ordinary passage through the room or adjustment of the apparatus. The effects of disturbances upon the activity of an otherwise quiescent fish may be seen in Figure 3. The room was entered several times in the course of the afternoon of July 3 and on July 5, although the experimental chamber was not approached and the fish could not see the cause of the disturbance. It is obvious that the rates of activity were higher than at corresponding hours on July 4, when the room was not entered. Such sensitivity has been observed in other species of fish by Clausen (1934), who found that a shadow passing over the aquarium caused increases in the body temperatures of perch and members of the sunfish group.

The Relationship Between Activity and Oxygen Consumption

Method

The activity and corresponding oxygen consumption of individual goldfish were measured in observation periods ranging in length from 11 to 210 minutes. Activity was measured in terms of the number of impulses recorded in a given period, and the amount of oxygen consumed by the fish in that period was determined by means of a continuous flow system. A control chamber similar to the experimental and housed in the same case was supplied with a continuous stream of water from the reservoir supplying the fish. The water in each chamber was covered with a layer of heavy paraffin oil 2.5 cm. thick to retard the diffusion of oxygen from the air, and a sample of the effluent from each chamber was analyzed for oxygen by the Winkler method at the beginning and end of each period. The samples were collected in narrow necked glass stoppered bottles of about 270 cc. capacity arranged to serve as constant level drains (Fig. 1). Each line was arranged so that the water passed through the outlet siphon to the bottom of the sampling bottle and overflowed into a funnel so that it could be collected for flow rate determinations. Although the rates of flow ranged from 70 to 100 cc. a minute in the course of the study, the rate for any one day's series of samples was held practically constant. Due care was taken to prevent the diffusion of oxygen into the samples and to obtain representative samples from experimental and control lines. Samples that were contaminated by particulate matter were discarded. The permanganate modification was used in most of the analyses, but was omitted during some of the shorter periods. The results obtained with and without the modification were quite similar, however, which was not unexpected in view of the fact that from four to six liters of water passed over the fish each hour.

The volume of water flowing through the system in the course of an observation period being known, together with the oxygen content of the water leaving the control and experimental chambers at the beginning and end of that period, the oxygen consumed by the fish could be calculated. The calculations took into account the change in the amount of oxygen in the constant volume of water in the chamber. The volume of water displaced by the fish was too small to affect the calculations.

The samples were collected with the foregoing observations on activity patterns

and modifying factors in mind, the periods being timed to yield data at the activity rates desired, and the method of sampling being modified as necessary to minimize disturbance of the fish. In the latter connection the outlet tubes were lengthened so that samples were collected about 10 feet away from the chambers, and the room was not entered except for sampling and rate of flow determinations. Precautions were taken to prevent changes in the water level in the experimental chamber as there were indications that small changes in the level stimulated the fish. These precautions were necessary also because the volume of water in the chamber, as well as that flowing through it, entered into the calculations of oxygen consumption. Samples were discarded if subsequent examinations of the activity records showed that they had been collected while the fish was undergoing marked changes in activity as a result of disturbance or in accordance with an activity rhythm. This was necessary because although the activity record was instantaneous the change in the oxygen concentration of the samples tended to lag somewhat behind that in the chamber, the sample drawn at any instant representing the average of the water flowing into the bottle in the few minutes preceding its removal. The temperature of the water was recorded for each observation period in order to avoid discrepancies attributable to the effect of temperature on metabolic rate (Ege and Krogh, 1914).

Owing to its viscosity and the accumulation of emulsified oil and water at the oil-water interface, the layer of oil interfered with the movements of the paddle shaft. Its thickness was therefore reduced to 1 cm. within the central glass tube, thus permitting the paddle to move about as freely as with a water surface. This tube extended below the interface far enough to prevent the emulsion from accumulating around the paddle shaft. The oil within the tube had to be changed now and then, however, to remove the small amount of debris that entered it from beneath. The detector contacts and bakelite rod were cleaned every few days as a precaution against their becoming coated with oil, which seemed to spread slowly up the paddle shaft.

It was established by appropriate tests that the layer of paraffin oil was effective in preventing the diffusion of significant amounts of oxygen into the water from the atmosphere. In no test did the apparent leakage exceed the limits of error of the Winkler method itself (Allee and Oesting, 1934), and it was usually considerably less. The average apparent rate of change for the contents of the experimental chamber was 0.0015 cc. of oxygen a minute, which was so much smaller than the rate at which the fish consumed oxygen that even had the apparent change been real it would have had but little effect on the results. It should be mentioned in this connection that the oil layer was disturbed relatively little by the movements of the fish, inasmuch as the wire screen kept the fish out of the oil and glass plates resting on this screen lessened the churning effects of the water beneath it.

Results

Three goldfish were studied at several temperatures in a total of 104 observation periods. As the results on all three were much alike, data on but one of the fish, a 32-gram male on which over two-thirds of the measurements were made, are presented here.

The relationship between activity and oxygen consumption at temperatures between 23 and 25° C. is shown in Figure 4, in which oxygen consumption in cubic

centimeters per minute is plotted against activity in impulses per minute. Fifty-nine observation periods are represented, each point corresponding to one period. The line merely indicates the trend, and has not been fitted to the data mathematically. As was to be expected, oxygen consumption and activity proved to be closely related, the relationship apparently being linear above the basal level of oxygen consumption. Although the values for oxygen consumption at any one rate of activity are seen to vary somewhat, the trend is clear cut: at high rates of activity the rate of oxygen

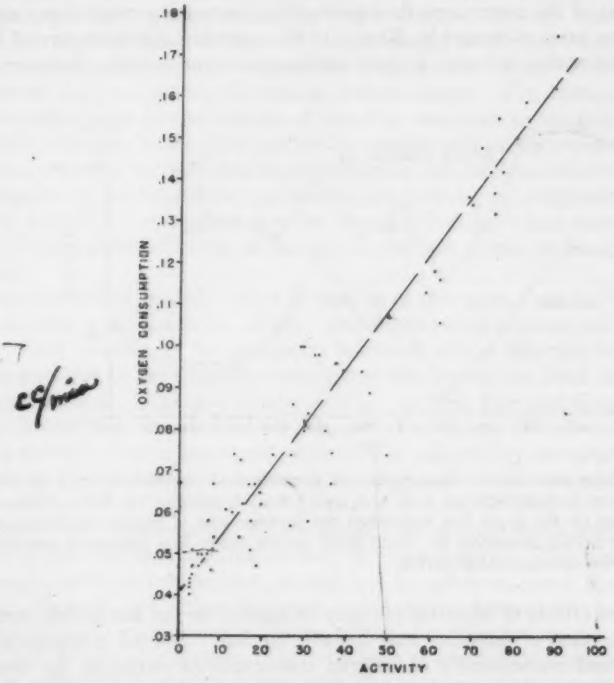


FIGURE 4. Activity and oxygen consumption of 32-gram male goldfish. Activity in impulses/minute; oxygen consumption in cubic centimeters/minute. Temperature 23 to 25° C.

consumption is correspondingly high; at low activity rates less oxygen is consumed. The discrepancies that do occur may well have been due to errors in measurement, rather than to a lack of correspondence between activity and oxygen consumption. In this connection the data on oxygen consumption follow those on activity quite closely when the comparison is restricted to one day's series of measurements, thus ruling out discrepancies attributable to slight differences in the adjustment of the detector contacts. Such a series is shown in Figure 5, which is based on data obtained with the same fish in a series of thirteen consecutive 15- to 25-minute periods at 22° C.

According to the slope of the data shown in Figure 4, the basal oxygen con-

sumption of this fish was in the vicinity of 0.040 cc. a minute, or 0.075 cc. per gram per hour.

Discussion

The results of the present study have a bearing on the collection and interpretation of data on the respiratory metabolism of fishes, and in the light of these results the method described seems to offer a number of advantages not found in previous methods which have been employed for this purpose.

The advantages of the continuous flow method for measuring respiratory metabolism in fishes have been discussed by Keys (1930) and need not be reviewed here. In view of the relationship between oxygen consumption and activity, however, the

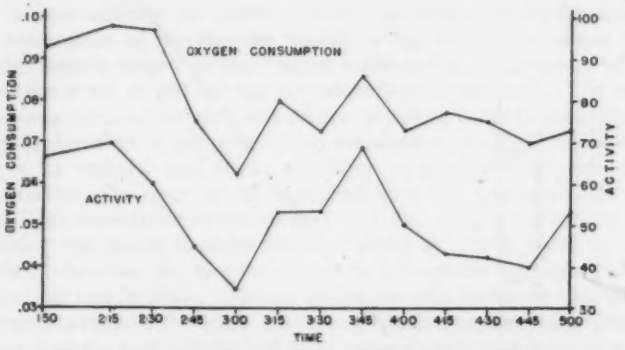


Figure 5. Activity and oxygen consumption of 32-gram male goldfish in each of thirteen consecutive observation periods between 1:35 P.M. and 5 P.M. November 13, 1945. Units as in Figure 4. Each point on the upper line represents the average rate of oxygen consumption for the 15- or 25-minute period preceding it. Each point on the lower line represents the average rate of activity for the corresponding period.

observations on the effects of disturbances may be applied to the use of this method, inasmuch as the process of sampling may disturb the fish. Should a change in the rate of activity (and consequently of oxygen consumption) occur at the time of sampling, the sample would not be representative of the volume of water and unit of time to which it is related in the calculations. The resulting error could be of considerable importance, particularly in investigations in which the samples consisted of water flowing directly from the experimental chamber and overflowing through a sampling bottle. This source of error has been recognized of course, and in some investigations the experimental chamber has been covered in attempts to minimize stimulation of the fish. It seems very doubtful, however, whether covering a goldfish so that it cannot see the investigator is an adequate safeguard against disturbance. One advantage of using an activity detector in the continuous flow method then lies in the fact that any sudden change in activity occurring at the time of sampling can be detected, so that the reliability of the sample may be judged. Furthermore, the activity record can be used to test the effectiveness of the steps taken to avoid disturbance.

It is of course well known that fluctuations in activity during the test periods constitute a major obstacle to the correct interpretation of measurements of oxygen consumption, and numerous attempts to overcome this difficulty have been described (Ege and Krogh, 1914; Hall, 1929; Adkins, 1930; Keys, 1930; Wells, 1932, 1935; Clausen, 1933; Smith and Matthews, 1942). These measures include the use of narcotics, observing that the fish remains quiet, maintaining constant conditions of light, sampling at the same time each day, restricting the movements of the fish, and maintaining the fish in an experimental chamber until it appears to have come to rest or at any rate to have reached a steady state. Although such measures may permit the establishment of the reality of a change in oxygen consumption in connection with an experimental procedure, they do not appear to give a completely satisfactory basis for the interpretation of that change. The interpretation must be based on knowledge of the activity of the fish, inasmuch as oxygen consumption is affected by changes in the basal metabolic rate as well as by activity. The method employed must therefore be capable of supplying information on activity and oxygen consumption at the same time, so that the fraction of the respiratory exchange associated with basal metabolism may be distinguished from that due to muscular activity (Wells, 1935). None of the above methods seems to be adequate for this purpose.

Narcotics are of doubtful value in studies of this type, even for measuring basal metabolic rate alone (Adkins, 1930). Among other objections are indications that an important fraction of the metabolic functions of the fish may be suppressed to such an extent that the oxygen consumption falls below the basal level as it is generally understood (Keys and Wells, 1930). In fact, Ege and Krogh (1914) considered it necessary to use artificial respiration to insure the survival of their goldfish, the narcotic having interfered with normal respiratory movements. The other methods are open to criticism because they are based on the assumption, rather than the knowledge, that the fish is quiescent or at a constant level of activity under the conditions of the experiment. The results of the present work suggest that for the goldfish at any rate this assumption may be unwarranted. The fact that a goldfish is quiescent while it can be seen should not be taken as proof that it remains so while unobserved, and it does not seem justifiable to assume that constant environmental conditions mean constant rates of activity. So far as the goldfish is concerned, the individual variations in activity open to question the reliability of methods based on sampling at the same time each day, particularly if several fish are being compared. Confining the fish to a small respiration chamber to restrict its movements gives no assurance that it will remain quiescent or even at a constant rate of activity, and the fact that the oxygen consumption varies over a wide range in such chambers supports this objection. This method would seem to have a further disadvantage for measuring the basal metabolic rate in that a fish confined to a small tube must swim continuously, however slowly, in order to maintain its position in the current. The practice of leaving the fish in the respiratory chamber until its oxygen consumption has reached a relatively low and constant rate (Keys, 1930; Wells, 1935) is far superior to the earlier techniques, but it is limited in its application by the fact that it gives no information as to the amount of activity associated with the steady state.

The requirements of a satisfactory method appear to be met by combining an activity detector with the continuous flow system. The rate of activity can then be

measured at the same time that oxygen consumption is determined, and the results interpreted accordingly. As the activity record is continuous, periods of quiescence can be selected for measuring basal oxygen consumption, so that it is not necessary to employ special techniques designed to control activity. In this connection, however, starvation may be used as a means of prolonging the quiescent state. A further advantage of the present method lies in the fact that the fish can be maintained in good health in the experimental chamber for months, so that measurements of its respiratory metabolism need not be obscured by the excitement and other effects of handling.

Summary

1. Apparatus for making continuous records of the activity of isolated and undisturbed goldfish is described, together with a method for measuring oxygen consumption and activity simultaneously.

2. The goldfish were quite variable in their patterns and rates of activity under the experimental conditions. Some fish were diurnally active and nocturnally quiescent, others followed the opposite pattern and still others were arhythmic throughout the periods during which they were observed. Moreover, some fish showed both rhythmic and arhythmic states of activity when studied for periods extending over several weeks or months.

3. Food, light and minor disturbances had pronounced effects on the activity of the goldfish.

4. Simultaneous measurements of oxygen consumption and activity are presented which indicate that the two are closely related above the basal level of oxygen consumption.

5. The bearing of these observations on the collection and interpretation of data on the oxygen consumption of the goldfish and on the measurement of its basal metabolic rate is discussed, and certain advantages of the method are described.

LITERATURE CITED

Adkins, M., 1930. A method for determining basal metabolism of fishes. *Proc. Soc. Exp. Biol. Med.*, **28**: 259–263.

Allee, W. C. and R. Oesting, 1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. *Physiol. Zool.*, **7**: 509–541.

Bowen, E. S., 1932. Further studies of the aggregating behavior of Ameiurus melas. *Biol. Bull.*, **63**: 258–270.

Clausen, R. G., 1933. Fish metabolism under increasing temperature. *Trans. Amer. Fish. Soc.*, **63**: 215–219.

Clausen, R. G., 1934. Body temperature of fresh water fishes. *Ecology*, **15**: 139–144.

Clausen, R. G., 1936. Oxygen consumption in fresh water fishes. *Ecology*, **17**: 216–226.

Crozier, W. J. and T. B. Stier, 1925. Critical increment for opercular breathing rhythm of the goldfish. *Jour. Gen. Physiol.*, **7**: 699–704.

Ege, R. and A. Krogh, 1914. On the relation between the temperature and the respiratory exchange in fishes. *Internat. Revue d. ges. Hydrobiol. u. Hydrog.*, **7**: 48–55.

Hall, F. G., 1929. The influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes. *Amer. Jour. Physiol.*, **88**: 212–218.

Keys, A. B., 1930. The measurement of the respiratory exchange of aquatic animals. *Biol. Bull.*, **59**: 187–198.

Keys, A. B. and N. A. Wells, 1930. Amytal anesthesia in fishes. *Jour. Pharm. Exp. Therap.*, **40**: 115–128.

KROGH, A., 1916. *The respiratory exchange of animals and man. Monographs on Biochemistry.* Longmans, Green and Co., London.

SCHLAIFER, A., 1938. Studies in mass physiology: effect of numbers upon the oxygen consumption and locomotor activity of Carassius auratus. *Physiol. Zool.*, **11**: 408–424.

SMITH, D. C. AND S. A. MATTHEWS, 1942. The effect of adrenalin on the oxygen consumption of the fish, Girella nigricans. *Amer. Jour. Physiol.*, **137**: 533–538.

SPENCER, W. P., 1939. Diurnal activity rhythms in fresh-water fishes. *Ohio Jour. Sci.*, **39**: 119–132.

SPOOR, W. A., 1941. A method for measuring the activity of fishes. *Ecology*, **22**: 329–331.

SZYMANSKI, J. S., 1914. Eine Methode zur Untersuchung der Ruhe- und Aktivitätsperioden bei Tieren. *Pflüger's Arch. ges. Physiol.*, **158**: 343–385.

TORYU, Y., 1927. The respiratory exchange in Carassius auratus and the gaseous exchange of the air bladder. *Sci. Rept. Tohoku Imp. Univ.*, 4 Ser. (Biology), **3**: 87–96.

WELLS, N. A., 1932. The importance of the time element in the determination of the respiratory metabolism of fishes. *Proc. Nat. Acad. Sci.*, **18**: 580–585.

WELLS, N. A., 1935. The influence of temperature upon the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis. *Physiol. Zool.*, **8**: 196–227.

INDEX

ABSTRACTS of scientific papers presented at the Marine Biological Laboratory, summer of 1946, 210.

Activity and oxygen consumption of the goldfish and its application to the measurement of respiratory metabolism in fishes, 312.

Amaroecium constellatum, II, 66.

Annual report of the Marine Biological Laboratory, 1.

Arbacia eggs, the effect of low temperature and of hypotonicity on the morphology of the cleavage furrow, 272.

Artemia salina, the space-time pattern of segment formation in, 119.

BEERS, C. D. Tillina magna: Micronuclear number, encystment and vitality in diverse clones; capabilities of amicronucleate races, 256.

BODENSTEIN, DIETRICH. Developmental relations between genital ducts and gonads in Drosophila, 288.

BROWN, FRANK A., JR., AND LORRAINE M. SAIGH. The comparative distribution of two chromatophorotropic hormones (CDH and CBLH) in Crustacean nervous systems, 170.

CARRIKER, MELBOURNE ROMAINE. Observations on the functioning of the alimentary system of the snail Lymnaea stagnalis appressa Say, 88.

CHEN, TZE-TUAN. Temporary pair formation in Paramecium bursaria, 112.

Chromosome movement, hydrostatic pressure effects upon the spindle figure and, 145.

Ciliates of the family Ancistrocomidae Chatton and Lwoff, III, 189.

Ciliates of the family Ancistrocomidae Chatton and Lwoff, IV, 200.

Cleavage furrow in Arbacia eggs, 272.

DDT, loci of action in cockroach (Periplaneta americana), 247.

Drosophila, developmental relations between genital ducts and gonads in, 288.

ELECTRON microscope observations of the trichocysts and cilia of Paramecium, 141.

GIESE, ARTHUR C. Comparative sensitivity of sperm and eggs to ultraviolet radiations, 81.

HABROBRACON, a strongly intersexual female in, 243.

HALL, C. E. *See* M. A. JAKUS, 141.

Histological study of Syndisyrinx franciscanus, an endoparasitic rhabdocoel of the sea urchin, 295

Hormones (CDH and CBLH) in Crustacean nervous systems, the comparative distribution of two chromatophorotropic, 170.

INFLUENCE of texture and composition of surface on the attachment of sedentary marine organisms, 57.

JAKUS, M. A., AND C. E. HALL. Electron microscope observations of trichocysts and cilia of Paramecium, 141.

KOLLROS, J. J. *See* J. M. TOBIAS, 247.

KOZLOFF, EUGENE N. Studies on ciliates of the family Ancistrocomidae Chatton and Lwoff (order Holotricha, suborder Thigmotricha). III. Ancistrocoma pelseneeri Chatton and Lwoff, Ancistrocoma dissimilis sp. nov., and Hypocomagalma pholadidis sp. nov., 189.

KOZLOFF, EUGENE N. Studies on ciliates of the family Ancistrocomidae Chatton and Lwoff (order Holotricha, suborder Thigmotricha). IV. Heterocineta janickii Jarocki, Heterocineta goniobasidis sp. nov., Heterocineta fluminicolae sp. nov., and Enerthecoma properans Jarocki, 200.

LEHMAN, H. E. A histological study of Syndisyrinx franciscanus, gen. et sp. nov., an endoparasitic rhabdocoel of the sea urchin, Strongylocentrotus franciscanus, 295.

Lymnaea stagnalis appressa Say, observations on the functioning of the alimentary system of the snail, 88.

MARINE Biological Laboratory, annual report, 1.

Marine Biological Laboratory, program and abstracts of scientific papers presented, summer of 1946, 210.

MORRISON, PETER R. Physiological observations on water loss and oxygen consumption in Peripatus, 181.

OXYGEN consumption and water loss in Peripatus, 181.
Oxygen consumption and activity of the goldfish, its application to the measurement of respiratory metabolism in fishes, 312.

PARAMECIUM bursaria, temporary pair formation in, 112.
Paramecium, electron microscope observations of the trichocysts and cilia of, 141.
PEASE, DANIEL C. Hydrostatic pressure effects upon the spindle figure and chromosome movement. II. Experiments on the meiotic divisions of Tradescantia pollen mother cells, 145.
POMERAT, C. M., AND C. M. WEISS. The influence of texture and composition of surface on the attachment of sedentary marine organisms, 57.

SAIGH, LORRAINE M. *See* FRANK A. BROWN, JR., 170.
SCOTT, ALLAN. The effect of low temperature and of hypotonicity on the morphology of the cleavage furrow in Arbacia eggs, 272.
SCOTT, SISTER FLORENCE MARIE. The developmental history of Amaroecium constellatum. II. Organogenesis of the larval action system, 66.
Society of General Physiologists, papers presented at the meeting of, 236.
Spindle figure and chromosome movement, hydrostatic pressure effects upon, 145.
SPOOR, W. A. A quantitative study of the relationship between the activity and oxygen consumption of the goldfish, and its application to the measurement of respiratory metabolism in fishes, 312.
Studies on ciliates of the family Ancistrocomidae Chatton and Lwoff (order Holotricha, suborder Thigmotricha). III. Ancistrocoma pelseneeri Chatton and Lwoff, Ancistrocoma dissimilis sp. nov., and Hypocomagalma pholadidis sp. nov., 189.
Studies on ciliates of the family Ancistrocomidae Chatton and Lwoff (order Holotricha, suborder Thigmotricha). IV. Heterocineta janickii Jarocki, Heterocineta goniobasidis sp. nov., Heterocineta fluminicolae sp. nov., and Enerthecoma properans Jarocki, 200.

TILLINA magna: Micronuclear number, encystment and vitality in diverse clones; capabilities of amicronucleate races, 256.
TOBIAS, J. M., AND J. J. KOLLROS. Loci of action of DDT in cockroach (Periplaneta americana), 247.

ULTRAVIOLET radiations, comparative sensitivity of sperm and eggs to, 81.

WEISS, C. M. *See* C. M. POMERAT, 57.
WEISZ, PAUL B. The space-time pattern of segment formation in Artemia salina, 119.
WHITING, P. W. A strongly intersexual female in Habrobracon, 243.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology, and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

Supply Department

MARINE BIOLOGICAL LABORATORY

Woods Hole, Massachusetts

CONTENTS

Page

WHITING, P. W.
A strongly intersexual female in Habrobracon 243

TOBIAS, J. M., AND J. J. KOLLROS
Loci of action of DDT in the cockroach (Periplaneta americana) 247

BEERS, C. D.
Tillina magna: Micronuclear number, encystment and vitality in diverse clones; capabilities of amicronucleate races 256

SCOTT, ALLAN
The effect of low temperature and of hypotonicity on the morphology of the cleavage furrow in Arbacia eggs 272

BODENSTEIN, DIETRICH
Developmental relations between genital ducts and gonads in Drosophila 288

LEHMAN, H. E.
A histological study of Syndisyrinx franciscanus, gen. et sp. nov., an endoparasitic rhabdocoel of the sea urchin, Strongylocentrotus franciscanus 295

SPOOR, W. A.
A quantitative study of the relationship between the activity and oxygen consumption of the goldfish, and its application to the measurement of respiratory metabolism in fishes 312